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(54) Title: COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

(57) Abstract: The present invention provides oligonucleotide primers and compositions and kits containing the same for rapid identification of bacteria by amplification of a segment of bacterial nucleic acid followed by molecular mass analysis.

## COMPOSITIONS FOR USE IN IDENTIFICATION OF BACTERIA

### CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] The present application claims the benefit of priority to: U.S. Provisional Application Serial No. 60/545,425 filed February 18, 2004, U.S. Provisional Application Serial No. 60/559,754, filed April 5, 2004, U.S. Provisional Application Serial No. 60/632,862, filed December 3, 2004, U.S. Provisional Application Serial No. 60/639,068, filed December 22, 2004, and U.S. Provisional Application Serial No. 60/648,188, filed January 28, 2005, each of which is incorporated herein by reference in its entirety.

### STATEMENT OF GOVERNMENT SUPPORT

[0002] This invention was made with United States Government support under DARPA/SPO contract BAA00-09. The United States Government may have certain rights in the invention.

### FIELD OF THE INVENTION

[0003] The present invention relates generally to the field of genetic identification of bacteria and provides nucleic acid compositions and kits useful for this purpose when combined with molecular mass analysis.

### BACKGROUND OF THE INVENTION

[0004] A problem in determining the cause of a natural infectious outbreak or a bioterrorist attack is the sheer variety of organisms that can cause human disease. There are over 1400 organisms infectious to humans; many of these have the potential to emerge suddenly in a natural epidemic or to be used in a malicious attack by bioterrorists (Taylor et al. Philos. Trans. R. Soc. London B. Biol. Sci., 2001, 356, 983-989). This number does not include numerous strain variants, bioengineered versions, or pathogens that infect plants or animals.

[0005] Much of the new technology being developed for detection of biological weapons incorporates a polymerase chain reaction (PCR) step based upon the use of highly specific primers and probes designed to selectively detect certain pathogenic organisms. Although this approach is appropriate for the most obvious bioterrorist organisms, like smallpox and anthrax, experience has shown that it is very difficult to predict which of hundreds of possible pathogenic organisms might be employed in a terrorist attack. Likewise, naturally emerging human disease that has caused devastating consequence in public health has come from unexpected families of

bacteria, viruses, fungi, or protozoa. Plants and animals also have their natural burden of infectious disease agents and there are equally important biosafety and security concerns for agriculture.

**[0006]** A major conundrum in public health protection, biodefense, and agricultural safety and security is that these disciplines need to be able to rapidly identify and characterize infectious agents, while there is no existing technology with the breadth of function to meet this need. Currently used methods for identification of bacteria rely upon culturing the bacterium to effect isolation from other organisms and to obtain sufficient quantities of nucleic acid followed by sequencing of the nucleic acid, both processes which are time and labor intensive.

**[0007]** Mass spectrometry provides detailed information about the molecules being analyzed, including high mass accuracy. It is also a process that can be easily automated. DNA chips with specific probes can only determine the presence or absence of specifically anticipated organisms. Because there are hundreds of thousands of species of benign bacteria, some very similar in sequence to threat organisms, even arrays with 10,000 probes lack the breadth needed to identify a particular organism.

**[0008]** There is a need for a method for identification of bioagents which is both specific and rapid, and in which no culture or nucleic acid sequencing is required. Disclosed in U.S. Patent Application Serial Nos: 09/798,007, 09/891,793, 10/405,756, 10/418,514, 10/660,997, 10/660,122, 10/660,996, 10/728,486, 10/754,415 and 10/829,826, each of which is commonly owned and incorporated herein by reference in its entirety, are methods for identification of bioagents (any organism, cell, or virus, living or dead, or a nucleic acid derived from such an organism, cell or virus) in an unbiased manner by molecular mass and base composition analysis of "bioagent identifying amplicons" which are obtained by amplification of segments of essential and conserved genes which are involved in, for example, translation, replication, recombination and repair, transcription, nucleotide metabolism, amino acid metabolism, lipid metabolism, energy generation, uptake, secretion and the like. Examples of these proteins include, but are not limited to, ribosomal RNAs, ribosomal proteins, DNA and RNA polymerases, elongation factors, tRNA synthetases, protein chain initiation factors, heat shock protein groEL, phosphoglycerate kinase, NADH dehydrogenase, DNA ligases, DNA gyrases and DNA topoisomerases, metabolic enzymes, and the like.

**[0009]** To obtain bioagent identifying amplicons, primers are selected to hybridize to conserved sequence regions which bracket variable sequence regions to yield a segment of nucleic acid which can be amplified and which is amenable to methods of molecular mass analysis. The variable sequence regions provide the variability of molecular mass which is used for bioagent identification. Upon amplification by PCR or other amplification methods with the specifically chosen primers, an amplification product that represents a bioagent identifying amplicon is obtained. The molecular mass of the amplification product, obtained by mass spectrometry for example, provides the means to uniquely identify the bioagent without a requirement for prior knowledge of the possible identity of the bioagent. The molecular mass of the amplification product or the corresponding base composition (which can be calculated from the molecular mass of the amplification product) is compared with a database of molecular masses or base compositions and a match indicates the identity of the bioagent. Furthermore, the method can be applied to rapid parallel analyses (for example, in a multi-well plate format) the results of which can be employed in a triangulation identification strategy which is amenable to rapid throughput and does not require nucleic acid sequencing of the amplified target sequence for bioagent identification.

**[0010]** The result of determination of a previously unknown base composition of a previously unknown bioagent (for example, a newly evolved and heretofore unobserved bacterium or virus) has downstream utility by providing new bioagent indexing information with which to populate base composition databases. The process of subsequent bioagent identification analyses is thus greatly improved as more base composition data for bioagent identifying amplicons becomes available.

**[0011]** The present invention provides oligonucleotide primers and compositions and kits containing the oligonucleotide primers, which define bacterial bioagent identifying amplicons and, upon amplification, produce corresponding amplification products whose molecular masses provide the means to identify bacteria, for example, at and below the species taxonomic level.

## **SUMMARY OF THE INVENTION**

**[0012]** The present invention provides primers and compositions comprising pairs of primers, and kits containing the same for use in identification of bacteria. The primers are designed to produce bacterial bioagent identifying amplicons of DNA encoding genes essential to life such as, for example, 16S and 23S rRNA, DNA-directed RNA polymerase subunits (rpoB and rpoC),



valyl-tRNA synthetase (valS), elongation factor EF-Tu (TufB), ribosomal protein L2 (rplB), protein chain initiation factor (infB), and spore protein (sspE). The invention further provides drill-down primers, compositions comprising pairs of primers and kits containing the same, which are designed to provide sub-species characterization of bacteria.

**[0013]** In particular, the present invention provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 80% to 100% sequence identity with SEQ ID NO: 26, or a composition comprising the same; an oligonucleotide primer 20 to 27 nucleobases in length comprising at least a 20 nucleobase portion of SEQ ID NO: 388, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 15 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 26, and a second oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 388.

**[0014]** The present invention also provides an oligonucleotide primer 22 to 35 nucleobases in length comprising SEQ ID NO: 29, or a composition comprising the same; an oligonucleotide primer 18 to 35 nucleobases in length comprising SEQ ID NO: 391, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 29, and a second oligonucleotide primer 13 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 391.

**[0015]** The present invention also provides an oligonucleotide primer 22 to 26 nucleobases in length comprising SEQ ID NO: 37, or a composition comprising the same; an oligonucleotide primer 20 to 30 nucleobases in length comprising SEQ ID NO: 362, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 37, and a second oligonucleotide primer 14 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 362.

**[0016]** The present invention also provides an oligonucleotide primer 13 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 48, or a composition comprising the same; an oligonucleotide primer 19 to 35 nucleobases in length comprising SEQ ID NO: 404, or a composition comprising the same; a composition comprising both primers; and

a composition comprising a first oligonucleotide primer 13 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 48, and a second oligonucleotide primer 14 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 404.

**[0017]** The present invention also provides an oligonucleotide primer 21 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 160, or a composition comprising the same; an oligonucleotide primer 21 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 515, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 21 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 160, and a second oligonucleotide primer 21 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 515.

**[0018]** The present invention also provides an oligonucleotide primer 17 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 261, or a composition comprising the same; an oligonucleotide primer 18 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 624, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 17 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 261, and a second oligonucleotide primer 18 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 624.

**[0019]** The present invention also provides an oligonucleotide primer 21 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 231, or a composition comprising the same; an oligonucleotide primer 17 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 591; , or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 21 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 231, and a second oligonucleotide primer 17 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 591.

**[0020]** The present invention also provides an oligonucleotide primer 14 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 349, or a composition

comprising the same; an oligonucleotide primer 17 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 711, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 14 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 349, and a second oligonucleotide primer 17 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 711.

**[0021]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 240, or a composition comprising the same; an oligonucleotide primer 15 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 596, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 240, and a second oligonucleotide primer 15 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 596.

**[0022]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 58, or a composition comprising the same; an oligonucleotide primer 21 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 414, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 58, and a second oligonucleotide primer 15 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 414.

**[0023]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 6, or a composition comprising the same; an oligonucleotide primer 16 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 369, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 6, and a second oligonucleotide primer 15 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 369.

**[0024]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 246, or a composition comprising the same; an oligonucleotide primer 19 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 602, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 246, and a second oligonucleotide primer 19 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 602.

**[0025]** The present invention also provides an oligonucleotide primer 21 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 256, or a composition comprising the same; an oligonucleotide primer 14 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 620, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 21 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 256, and a second oligonucleotide primer 14 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 620.

**[0026]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 344, or a composition comprising the same; an oligonucleotide primer 18 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 700, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 344, and a second oligonucleotide primer 18 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 700.

**[0027]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 235, or a composition comprising the same; an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 587, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of

SEQ ID NO: 235, and a second oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 587.

**[0028]** The present invention also provides an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 322, or a composition comprising the same; an oligonucleotide primer 19 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 686, or a composition comprising the same; a composition comprising both primers; and a composition comprising a first oligonucleotide primer 16 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 322, and a second oligonucleotide primer 19 to 35 nucleobases in length comprising between 70% to 100% sequence identity of SEQ ID NO: 686.

**[0029]** The present invention also provides compositions, such as those described herein, wherein either or both of the first and second oligonucleotide primers comprise at least one modified nucleobase, a non-templated T residue on the 5'-end, at least one non-template tag, or at least one molecular mass modifying tag, or any combination thereof.

**[0030]** The present invention also provides kits comprising any of the compositions described herein. The kits can comprise at least one calibration polynucleotide, or at least one ion exchange resin linked to magnetic beads, or both.

**[0031]** The present invention also provides methods for identification of an unknown bacterium. Nucleic acid from the bacterium is amplified using any of the compositions described herein to obtain an amplification product. The molecular mass of the amplification product is determined. Optionally, the base composition of the amplification product is determined from the molecular mass. The base composition or molecular mass is compared with a plurality of base compositions or molecular masses of known bacterial bioagent identifying amplicons, wherein a match between the base composition or molecular mass and a member of the plurality of base compositions or molecular masses identifies the unknown bacterium. The molecular mass can be measured by mass spectrometry. In addition, the presence or absence of a particular clade, genus, species, or sub-species of a bioagent can be determined by the methods described herein.

**[0032]** The present invention also provides methods for determination of the quantity of an unknown bacterium in a sample. The sample is contacted with any of the compositions described

herein and a known quantity of a calibration polynucleotide comprising a calibration sequence. Concurrently, nucleic acid from the bacterium in the sample is amplified with any of the compositions described herein and nucleic acid from the calibration polynucleotide in the sample is amplified with any of the compositions described herein to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon. The molecular mass and abundance for the bacterial bioagent identifying amplicon and the calibration amplicon is determined. The bacterial bioagent identifying amplicon is distinguished from the calibration amplicon based on molecular mass, wherein comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of bacterium in the sample. The method can also comprise determining the base composition of the bacterial bioagent identifying amplicon.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0033]** Figure 1 is a representative pseudo-four dimensional plot of base compositions of bioagent identifying amplicons of enterobacteria obtained with a primer pair targeting the rpoB gene (primer pair no 14 (SEQ ID NOs: 37:362)). The quantity each of the nucleobases A, G and C are represented on the three axes of the plot while the quantity of nucleobase T is represented by the diameter of the spheres. Base composition probability clouds surrounding the spheres are also shown.

**[0034]** Figure 2 is a representative diagram illustrating the primer selection process.

**[0035]** Figure 3 lists common pathogenic bacteria and primer pair coverage. The primer pair number in the upper right hand corner of each polygon indicates that the primer pair can produce a bioagent identifying amplicon for all species within that polygon.

**[0036]** Figure 4 is a representative 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples (labeled NHRC samples) closely match the base compositions expected for *Streptococcus pyogenes* and are distinct from the expected base compositions of other organisms.

[0037] Figure 5 is a representative mass spectrum of amplification products representing bioagent identifying amplicons of *Streptococcus pyogenes*, *Neisseria meningitidis*, and *Haemophilus influenzae* obtained from amplification of nucleic acid from a clinical sample with primer pair number 349 which targets 23S rRNA. Experimentally determined molecular masses and base compositions for the sense strand of each amplification product are shown.

[0038] Figure 6 is a representative mass spectrum of amplification products representing a bioagent identifying amplicon of *Streptococcus pyogenes*, and a calibration amplicon obtained from amplification of nucleic acid from a clinical sample with primer pair number 356 which targets rplB. The experimentally determined molecular mass and base composition for the sense strand of the *Streptococcus pyogenes* amplification product is shown.

[0039] Figure 7 is a representative process diagram for identification and determination of the quantity of a bioagent in a sample.

[0040] Figure 8 is a representative mass spectrum of an amplified nucleic acid mixture which contained the Ames strain of *Bacillus anthracis*, a known quantity of combination calibration polynucleotide (SEQ ID NO: 741), and primer pair number 350 which targets the capC gene on the virulence plasmid pX02 of *Bacillus anthracis*. Calibration amplicons produced in the amplification reaction are visible in the mass spectrum as indicated and abundance data (peak height) are used to calculate the quantity of the Ames strain of *Bacillus anthracis*.

## DESCRIPTION OF EMBODIMENTS

[0041] The present invention provides oligonucleotide primers which hybridize to conserved regions of nucleic acid of genes encoding, for example, proteins or RNAs necessary for life which include, but are not limited to: 16S and 23S rRNAs, RNA polymerase subunits, t-RNA synthetases, elongation factors, ribosomal proteins, protein chain initiation factors, cell division proteins, chaperonin groEL, chaperonin dnaK, phosphoglycerate kinase, NADH dehydrogenase, DNA ligases, metabolic enzymes and DNA topoisomerases. These primers provide the functionality of producing, for example, bacterial bioagent identifying amplicons for general identification of bacteria at the species level, for example, when contacted with bacterial nucleic acid under amplification conditions.

[0042] Referring to Figure 2, primers are designed as follows: for each group of organisms, candidate target sequences are identified (200) from which nucleotide alignments are created (210) and analyzed (220). Primers are designed by selecting appropriate priming regions (230) which allows the selection of candidate primer pairs (240). The primer pairs are subjected to *in silico* analysis by electronic PCR (ePCR) (300) wherein bioagent identifying amplicons are obtained from sequence databases such as, for example, GenBank or other sequence collections (310), and checked for specificity *in silico* (320). Bioagent identifying amplicons obtained from GenBank sequences (310) can also be analyzed by a probability model which predicts the capability of a particular amplicon to identify unknown bioagents such that the base compositions of amplicons with favorable probability scores are stored in a base composition database (325). Alternatively, base compositions of the bioagent identifying amplicons obtained from the primers and GenBank sequences can be directly entered into the base composition database (330). Candidate primer pairs (240) are validated by *in vitro* amplification by a method such as, for example, PCR analysis (400) of nucleic acid from a collection of organisms (410). Amplification products that are obtained are optionally analyzed to confirm the sensitivity, specificity and reproducibility of the primers used to obtain the amplification products (420).

[0043] Synthesis of primers is well known and routine in the art. The primers may be conveniently and routinely made through the well-known technique of solid phase synthesis. Equipment for such synthesis is sold by several vendors including, for example, Applied Biosystems (Foster City, CA). Any other means for such synthesis known in the art may additionally or alternatively be employed.

[0044] The primers can be employed as compositions for use in, for example, methods for identification of bacterial bioagents as follows. In some embodiments, a primer pair composition is contacted with nucleic acid of an unknown bacterial bioagent. The nucleic acid is amplified by a nucleic acid amplification technique, such as PCR for example, to obtain an amplification product that represents a bioagent identifying amplicon. The molecular mass of one strand or each strand of the double-stranded amplification product is determined by a molecular mass measurement technique such as, for example, mass spectrometry wherein the two strands of the double-stranded amplification product are separated during the ionization process. In some embodiments, the mass spectrometry is electrospray Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS) or electrospray time of flight mass spectrometry (ESI-TOF-MS). A list of possible base compositions can be generated for the molecular mass value



obtained for each strand and the choice of the correct base composition from the list is facilitated by matching the base composition of one strand with a complementary base composition of the other strand. The molecular mass or base composition thus determined is compared with a database of molecular masses or base compositions of analogous bioagent identifying amplicons for known bacterial bioagents. A match between the molecular mass or base composition of the amplification product from the unknown bacterial bioagent and the molecular mass or base composition of an analogous bioagent identifying amplicon for a known bacterial bioagent indicates the identity of the unknown bioagent.

[0045] In some embodiments, the primer pair used is one of the primer pairs of Table 1. In some embodiments, the method is repeated using a different primer pair to resolve possible ambiguities in the identification process or to improve the confidence level for the identification assignment.

[0046] In some embodiments, a bioagent identifying amplicon may be produced using only a single primer (either the forward or reverse primer of any given primer pair), provided an appropriate amplification method is chosen, such as, for example, low stringency single primer PCR (LSSP-PCR). Adaptation of this amplification method in order to produce bioagent identifying amplicons can be accomplished by one with ordinary skill in the art without undue experimentation.

[0047] In some embodiments, the oligonucleotide primers are "broad range survey primers" which hybridize to conserved regions of nucleic acid encoding RNA, such as ribosomal RNA (rRNA), of all, or at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% of known bacteria and produce bacterial bioagent identifying amplicons. As used herein, the term "broad range survey primers" refers to primers that bind to nucleic acid encoding rRNAs of all, or at least 70%, at least 80%, at least 85%, at least 90%, or at least 95% known species of bacteria. In some embodiments, the rRNAs to which the primers hybridize are 16S and 23S rRNAs. In some embodiments, the broad range survey primer pairs comprise oligonucleotides ranging in length from 13 to 35 nucleobases, each of which have from 70% to 100% sequence identity with primer pair numbers 3, 10, 11, 14, 16, and 17 which consecutively correspond to SEQ ID NOs: 6:369, 26:388, 29:391, 37:362, 48:404, and 58:414.

[0048] In some cases, the molecular mass or base composition of a bacterial bioagent identifying amplicon defined by a broad range survey primer pair does not provide enough resolution to unambiguously identify a bacterial bioagent at the species level. These cases benefit from further analysis of one or more bacterial bioagent identifying amplicons generated from at least one additional broad range survey primer pair or from at least one additional “division-wide” primer pair (*vide infra*). The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as “triangulation identification” (*vide infra*).

[0049] In other embodiments, the oligonucleotide primers are “division-wide” primers which hybridize to nucleic acid encoding genes of broad divisions of bacteria such as, for example, members of the *Bacillus/Clostridia* group or members of the  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\epsilon$ -proteobacteria. In some embodiments, a division of bacteria comprises any grouping of bacterial genera with more than one genus represented. For example, the  $\beta$ -proteobacteria group comprises members of the following genera: *Eikenella*, *Neisseria*, *Achromobacter*, *Bordetella*, *Burkholderia*, and *Ralstonia*. Species members of these genera can be identified using bacterial bioagent identifying amplicons generated with primer pair 293 (SEQ ID NOs: 344:700) which produces a bacterial bioagent identifying amplicon from the *tufB* gene of  $\beta$ -proteobacteria. Examples of genes to which division-wide primers may hybridize to include, but are not limited to: RNA polymerase subunits such as *rpoB* and *rpoC*, tRNA synthetases such as valyl-tRNA synthetase (*valS*) and aspartyl-tRNA synthetase (*aspS*), elongation factors such as elongation factor EF-Tu (*tufB*), ribosomal proteins such as ribosomal protein L2 (*rplB*), protein chain initiation factors such as protein chain initiation factor *infB*, chaperonins such as *groL* and *dnaK*, and cell division proteins such as peptidase *ftsH* (*hflB*). In some embodiments, the division-wide primer pairs comprise oligonucleotides ranging in length from 13 to 35 nucleobases, each of which have from 70% to 100% sequence identity with primer pair numbers 34, 52, 66, 67, 71, 72, 289, 290 and 293 which consecutively correspond to SEQ ID NOs: 160:515, 261:624, 231:591, 235:587, 349:711, 240:596, 246:602, 256:620, 344:700.

[0050] In other embodiments, the oligonucleotide primers are designed to enable the identification of bacteria at the clade group level, which is a monophyletic taxon referring to a group of organisms which includes the most recent common ancestor of all of its members and all of the descendants of that most recent common ancestor. The *Bacillus cereus* clade is an example of a bacterial clade group. In some embodiments, the clade group primer pairs comprise oligonucleotides ranging in length from 13 to 35 nucleobases, each of which have from 70% to

100% sequence identity with primer pair number 58 which corresponds to SEQ ID NOs: 322:686.

[0051] In other embodiments, the oligonucleotide primers are “drill-down” primers which enable the identification of species or “sub-species characteristics.” Sub-species characteristics are herein defined as genetic characteristics that provide the means to distinguish two members of the same bacterial species. For example, *Escherichia coli* O157:H7 and *Escherichia coli* K12 are two well known members of the species *Escherichia coli*. *Escherichia coli* O157:H7, however, is highly toxic due to its Shiga toxin gene which is an example of a sub-species characteristic. Examples of sub-species characteristics may also include, but are not limited to: variations in genes such as single nucleotide polymorphisms (SNPs), variable number tandem repeats (VNTRs). Examples of genes indicating sub-species characteristics include, but are not limited to, housekeeping genes, toxin genes, pathogenicity markers, antibiotic resistance genes and virulence factors. Drill-down primers provide the functionality of producing bacterial bioagent identifying amplicons for drill-down analyses such as strain typing when contacted with bacterial nucleic acid under amplification conditions. Identification of such sub-species characteristics is often critical for determining proper clinical treatment of bacterial infections. Examples of pairs of drill-down primers include, but are not limited to, a trio of primer pairs for identification of strains of *Bacillus anthracis*. Primer pair 24 (SEQ ID NOs: 97:451) targets the capC gene of virulence plasmid pX02, primer pair 30 (SEQ ID NOs: 127:482) targets the cyA gene of virulence plasmid pX02, and primer pair 37 (SEQ ID NOs: 174:530) targets the lef gene of virulence plasmid pX02. Additional examples of drill-down primers include, but are not limited to, six primer pairs that are used for determining the strain type of group A *Streptococcus*. Primer pair 80 (SEQ ID NOs: 310:668) targets the gki gene, primer pair 81 (SEQ ID NOs: 313:670) targets the gtr gene, primer pair 86 (SEQ ID NOs: 227:632) targets the murI gene, primer pair 90 (SEQ ID NOs: 285:640) targets the mutS gene, primer pair 96 (SEQ ID NOs: 301:656) targets the xpt gene, and primer pair 98 (SEQ ID NOs: 308:663) targets the yqiL gene.

[0052] In some embodiments, the primers used for amplification hybridize to and amplify genomic DNA, DNA of bacterial plasmids, or DNA of DNA viruses.

[0053] In some embodiments, the primers used for amplification hybridize directly to ribosomal RNA or messenger RNA (mRNA) and act as reverse transcription primers for obtaining DNA from direct amplification of bacterial RNA or rRNA. Methods of amplifying RNA using reverse

transcriptase are well known to those with ordinary skill in the art and can be routinely established without undue experimentation.

[0054] One with ordinary skill in the art of design of amplification primers will recognize that a given primer need not hybridize with 100% complementarity in order to effectively prime the synthesis of a complementary nucleic acid strand in an amplification reaction. Moreover, a primer may hybridize over one or more segments such that intervening or adjacent segments are not involved in the hybridization event (e.g., a loop structure or a hairpin structure). The primers of the present invention may comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity with any of the primers listed in Table 1. Thus, in some embodiments of the present invention, an extent of variation of 70% to 100%, or any range therewithin, of the sequence identity is possible relative to the specific primer sequences disclosed herein. Determination of sequence identity is described in the following example: a primer 20 nucleobases in length which is otherwise identical to another 20 nucleobase primer but having two non-identical residues has 18 of 20 identical residues ( $18/20 = 0.9$  or 90% sequence identity). In another example, a primer 15 nucleobases in length having all residues identical to a 15 nucleobase segment of primer 20 nucleobases in length would have  $15/20 = 0.75$  or 75% sequence identity with the 20 nucleobase primer.

[0055] Percent homology, sequence identity or complementarity, can be determined by, for example, the Gap program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, Madison WI), using default settings, which uses the algorithm of Smith and Waterman (Adv. Appl. Math., 1981, 2, 482-489). In some embodiments, homology, sequence identity, or complementarity of primers with respect to the conserved priming regions of bacterial nucleic acid, is at least 70%, at least 80%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or is 100%.

[0056] In some embodiments, the primers described herein comprise at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 92%, at least 94%, at least 95%, at least 96%, at least 98%, or at least 99%, or 100% (or any range therewithin) sequence identity with the primer sequences specifically disclosed herein. Thus, for example, a primer may have between 70% and 100%, between 75% and 100%, between 80% and 100%, and between 95% and 100% sequence identity with SEQ ID NO: 26. Likewise, a primer may have similar sequence identity with any other primer whose nucleotide sequence is disclosed herein.

[0057] One with ordinary skill is able to calculate percent sequence identity or percent sequence homology and able to determine, without undue experimentation, the effects of variation of primer sequence identity on the function of the primer in its role in priming synthesis of a complementary strand of nucleic acid for production of an amplification product of a corresponding bioagent identifying amplicon.

[0058] In some embodiments of the present invention, the oligonucleotide primers are between 13 and 35 nucleobases in length (13 to 35 linked nucleotide residues). These embodiments comprise oligonucleotide primers 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 nucleobases in length, or any range therewithin.

[0059] In some embodiments, any given primer comprises a modification comprising the addition of a non-templated T residue to the 5' end of the primer (i.e., the added T residue does not necessarily hybridize to the nucleic acid being amplified). The addition of a non-templated T residue has an effect of minimizing the addition of non-templated A residues as a result of the non-specific enzyme activity of *Taq* polymerase (Magnuson et al. Biotechniques, 1996, 21, 700-709), an occurrence which may lead to ambiguous results arising from molecular mass analysis.

[0060] In some embodiments of the present invention, primers may contain one or more universal bases. Because any variation (due to codon wobble in the 3<sup>rd</sup> position) in the conserved regions among species is likely to occur in the third position of a DNA triplet, oligonucleotide primers can be designed such that the nucleotide corresponding to this position is a base which can bind to more than one nucleotide, referred to herein as a "universal nucleobase." For example, under this "wobble" pairing, inosine (I) binds to U, C or A; guanine (G) binds to U or C, and uridine (U) binds to U or C. Other examples of universal nucleobases include nitroindoles such as 5-nitroindole or 3-nitropyrrole (Loakes et al., Nucleosides and Nucleotides, 1995, 14, 1001-1003), the degenerate nucleotides dP or dK (Hill et al.), an acyclic nucleoside analog containing 5-nitroindazole (Van Aerschot et al., Nucleosides and Nucleotides, 1995, 14, 1053-1056) or the purine analog 1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide (Sala et al., Nucl. Acids Res., 1996, 24, 3302-3306).

[0061] In some embodiments, to compensate for the somewhat weaker binding by the "wobble" base, the oligonucleotide primers are designed such that the first and second positions of each triplet are occupied by nucleotide analogs which bind with greater affinity than the unmodified

nucleotide. Examples of these analogs include, but are not limited to, 2,6-diaminopurine which binds to thymine, 5-propynyluracil which binds to adenine and 5-propynylcytosine and phenoxazines, including G-clamp, which binds to G. Propynylated pyrimidines are described in U.S. Patent Nos. 5,645,985, 5,830,653 and 5,484,908, each of which is commonly owned and incorporated herein by reference in its entirety. Propynylated primers are described in U.S. Serial No. 10/294,203 which is also commonly owned and incorporated herein by reference in entirety. Phenoxazines are described in U.S. Patent Nos. 5,502,177, 5,763,588, and 6,005,096, each of which is incorporated herein by reference in its entirety. G-clamps are described in U.S. Patent Nos. 6,007,992 and 6,028,183, each of which is incorporated herein by reference in its entirety.

**[0062]** In some embodiments, non-template primer tags are used to increase the melting temperature ( $T_m$ ) of a primer-template duplex in order to improve amplification efficiency. A non-template tag is at least three consecutive A or T nucleotide residues on a primer which are not complementary to the template. In any given non-template tag, A can be replaced by C or G and T can also be replaced by C or G. Although Watson-Crick hybridization is not expected to occur for a non-template tag relative to the template, the extra hydrogen bond in a G-C pair relative to a A-T pair confers increased stability of the primer-template duplex and improves amplification efficiency for subsequent cycles of amplification when the primers hybridize to strands synthesized in previous cycles.

**[0063]** In other embodiments, propynylated tags may be used in a manner similar to that of the non-template tag, wherein two or more 5-propynylcytidine or 5-propynyluridine residues replace template matching residues on a primer. In other embodiments, a primer contains a modified internucleoside linkage such as a phosphorothioate linkage, for example.

**[0064]** In some embodiments, the primers contain mass-modifying tags. Reducing the total number of possible base compositions of a nucleic acid of specific molecular weight provides a means of avoiding a persistent source of ambiguity in determination of base composition of amplification products. Addition of mass-modifying tags to certain nucleobases of a given primer will result in simplification of *de novo* determination of base composition of a given bioagent identifying amplicon (*vide infra*) from its molecular mass.

**[0065]** In some embodiments of the present invention, the mass modified nucleobase comprises one or more of the following: for example, 7-deaza-2'-deoxyadenosine-5-triphosphate, 5-iodo-2'-

deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxyuridine-5'-triphosphate, 5-bromo-2'-deoxycytidine-5'-triphosphate, 5-iodo-2'-deoxycytidine-5'-triphosphate, 5-hydroxy-2'-deoxyuridine-5'-triphosphate, 4-thiothymidine-5'-triphosphate, 5-aza-2'-deoxyuridine-5'-triphosphate, 5-fluoro-2'-deoxyuridine-5'-triphosphate, O6-methyl-2'-deoxyguanosine-5'-triphosphate, N2-methyl-2'-deoxyguanosine-5'-triphosphate, 8-oxo-2'-deoxyguanosine-5'-triphosphate or thiothymidine-5'-triphosphate. In some embodiments, the mass-modified nucleobase comprises  $^{15}\text{N}$  or  $^{13}\text{C}$  or both  $^{15}\text{N}$  and  $^{13}\text{C}$ .

[0066] In some embodiments of the present invention, at least one bacterial nucleic acid segment is amplified in the process of identifying the bioagent. Thus, the nucleic acid segments that can be amplified by the primers disclosed herein and that provide enough variability to distinguish each individual bioagent and whose molecular masses are amenable to molecular mass determination are herein described as "bioagent identifying amplicons." The term "amplicon" as used herein, refers to a segment of a polynucleotide which is amplified in an amplification reaction. In some embodiments of the present invention, bioagent identifying amplicons comprise from about 45 to about 200 nucleobases (i.e. from about 45 to about 200 linked nucleosides), from about 60 to about 150 nucleobases, from about 75 to about 125 nucleobases. One of ordinary skill in the art will appreciate that the invention embodies compounds of 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, and 200 nucleobases in length, or any range therewithin. It is the combination of the portions of the bioagent nucleic acid segment to which the primers hybridize (hybridization sites) and the variable region between the primer hybridization sites that comprises the bioagent identifying amplicon. Since genetic data provide the underlying basis for identification of bioagents by the methods of the present invention, it is prudent to select segments of nucleic acids which ideally provide enough variability to distinguish each individual bioagent and whose molecular mass is amenable to molecular mass determination.

[0067] In some embodiments, bioagent identifying amplicons amenable to molecular mass determination which are produced by the primers described herein are either of a length, size or mass compatible with the particular mode of molecular mass determination or compatible with a means of providing a predictable fragmentation pattern in order to obtain predictable fragments of a length compatible with the particular mode of molecular mass determination. Such means of providing a predictable fragmentation pattern of an amplification product include, but are not limited to, cleavage with restriction enzymes or cleavage primers, for example. Methods of using restriction enzymes and cleavage primers are well known to those with ordinary skill in the art.

[0068] In some embodiments, amplification products corresponding to bacterial bioagent identifying amplicons are obtained using the polymerase chain reaction (PCR) which is a routine method to those with ordinary skill in the molecular biology arts. Other amplification methods may be used such as ligase chain reaction (LCR), low-stringency single primer PCR, and multiple strand displacement amplification (MDA) which are also well known to those with ordinary skill.

[0069] In the context of this invention, a “bioagent” is any organism, cell, or virus, living or dead, or a nucleic acid derived from such an organism, cell or virus. Examples of bioagents include, but are not limited, to cells, (including but not limited to human clinical samples, bacterial cells and other pathogens), viruses, fungi, protists, parasites, and pathogenicity markers (including but not limited to: pathogenicity islands, antibiotic resistance genes, virulence factors, toxin genes and other bioregulating compounds). Samples may be alive or dead or in a vegetative state (for example, vegetative bacteria or spores) and may be encapsulated or bioengineered. In the context of this invention, a “pathogen” is a bioagent which causes a disease or disorder.

[0070] In the context of this invention, the term “unknown bioagent” may mean either: (i) a bioagent whose existence is known (such as the well known bacterial species *Staphylococcus aureus* for example) but which is not known to be in a sample to be analyzed, or (ii) a bioagent whose existence is not known (for example, the SARS coronavirus was unknown prior to April 2003). For example, if the method for identification of coronaviruses disclosed in commonly owned U.S. Patent Serial No. 10/829,826 (incorporated herein by reference in its entirety) was to be employed prior to April 2003 to identify the SARS coronavirus in a clinical sample, both meanings of “unknown” bioagent are applicable since the SARS coronavirus was unknown to



science prior to April, 2003 and since it was not known what bioagent (in this case a coronavirus) was present in the sample. On the other hand, if the method of U.S. Patent Serial No. 10/829,826 was to be employed subsequent to April 2003 to identify the SARS coronavirus in a clinical sample, only the first meaning (i) of “unknown” bioagent would apply since the SARS coronavirus became known to science subsequent to April 2003 and since it was not known what bioagent was present in the sample.

[0071] The employment of more than one bioagent identifying amplicon for identification of a bioagent is herein referred to as “triangulation identification.” Triangulation identification is pursued by analyzing a plurality of bioagent identifying amplicons selected within multiple core genes. This process is used to reduce false negative and false positive signals, and enable reconstruction of the origin of hybrid or otherwise engineered bioagents. For example, identification of the three part toxin genes typical of *B. anthracis* (Bowen et al., J. Appl. Microbiol., 1999, 87, 270-278) in the absence of the expected signatures from the *B. anthracis* genome would suggest a genetic engineering event.

[0072] In some embodiments, the triangulation identification process can be pursued by characterization of bioagent identifying amplicons in a massively parallel fashion using the polymerase chain reaction (PCR), such as multiplex PCR where multiple primers are employed in the same amplification reaction mixture, or PCR in multi-well plate format wherein a different and unique pair of primers is used in multiple wells containing otherwise identical reaction mixtures. Such multiplex and multi-well PCR methods are well known to those with ordinary skill in the arts of rapid throughput amplification of nucleic acids.

[0073] In some embodiments, the molecular mass of a particular bioagent identifying amplicon is determined by mass spectrometry. Mass spectrometry has several advantages, not the least of which is high bandwidth characterized by the ability to separate (and isolate) many molecular peaks across a broad range of mass to charge ratio ( $m/z$ ). Thus, mass spectrometry is intrinsically a parallel detection scheme without the need for radioactive or fluorescent labels, since every amplification product is identified by its molecular mass. The current state of the art in mass spectrometry is such that less than femtomole quantities of material can be readily analyzed to afford information about the molecular contents of the sample. An accurate assessment of the molecular mass of the material can be quickly obtained, irrespective of whether the molecular

weight of the sample is several hundred, or in excess of one hundred thousand atomic mass units (amu) or Daltons.

[0074] In some embodiments, intact molecular ions are generated from amplification products using one of a variety of ionization techniques to convert the sample to gas phase. These ionization methods include, but are not limited to, electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI) and fast atom bombardment (FAB). Upon ionization, several peaks are observed from one sample due to the formation of ions with different charges. Averaging the multiple readings of molecular mass obtained from a single mass spectrum affords an estimate of molecular mass of the bioagent identifying amplicon. Electrospray ionization mass spectrometry (ESI-MS) is particularly useful for very high molecular weight polymers such as proteins and nucleic acids having molecular weights greater than 10 kDa, since it yields a distribution of multiply-charged molecules of the sample without causing a significant amount of fragmentation.

[0075] The mass detectors used in the methods of the present invention include, but are not limited to, Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS), time of flight (TOF), ion trap, quadrupole, magnetic sector, Q-TOF, and triple quadrupole.

[0076] In some embodiments, conversion of molecular mass data to a base composition is useful for certain analyses. As used herein, a “base composition” is the exact number of each nucleobase (A, T, C and G). For example, amplification of nucleic acid of *Neisseria meningitidis* with a primer pair that produces an amplification product from nucleic acid of 23S rRNA that has a molecular mass (sense strand) of 28480.75124, from which a base composition of A25 G27 C22 T18 is assigned from a list of possible base compositions calculated from the molecular mass using standard known molecular masses of each of the four nucleobases.

[0077] In some embodiments, assignment of base compositions to experimentally determined molecular masses is accomplished using “base composition probability clouds.” Base compositions, like sequences, vary slightly from isolate to isolate within species. It is possible to manage this diversity by building “base composition probability clouds” around the composition constraints for each species. This permits identification of organisms in a fashion similar to sequence analysis. A “pseudo four-dimensional plot” (Figure 1) can be used to visualize the concept of base composition probability clouds. Optimal primer design requires optimal choice

of bioagent identifying amplicons and maximizes the separation between the base composition signatures of individual bioagents. Areas where clouds overlap indicate regions that may result in a misclassification, a problem which is overcome by a triangulation identification process using bioagent identifying amplicons not affected by overlap of base composition probability clouds.

[0078] In some embodiments, base composition probability clouds provide the means for screening potential primer pairs in order to avoid potential misclassifications of base compositions. In other embodiments, base composition probability clouds provide the means for predicting the identity of a bioagent whose assigned base composition was not previously observed and/or indexed in a bioagent identifying amplicon base composition database due to evolutionary transitions in its nucleic acid sequence. Thus, in contrast to probe-based techniques, mass spectrometry determination of base composition does not require prior knowledge of the composition or sequence in order to make the measurement.

[0079] The present invention provides bioagent classifying information similar to DNA sequencing and phylogenetic analysis at a level sufficient to identify a given bioagent. Furthermore, the process of determination of a previously unknown base composition for a given bioagent (for example, in a case where sequence information is unavailable) has downstream utility by providing additional bioagent indexing information with which to populate base composition databases. The process of future bioagent identification is thus greatly improved as more BCS indexes become available in base composition databases.

[0080] In one embodiment, a sample comprising an unknown bioagent is contacted with a pair of primers which provide the means for amplification of nucleic acid from the bioagent, and a known quantity of a polynucleotide that comprises a calibration sequence. The nucleic acids of the bioagent and of the calibration sequence are amplified and the rate of amplification is reasonably assumed to be similar for the nucleic acid of the bioagent and of the calibration sequence. The amplification reaction then produces two amplification products: a bioagent identifying amplicon and a calibration amplicon. The bioagent identifying amplicon and the calibration amplicon should be distinguishable by molecular mass while being amplified at essentially the same rate. Effecting differential molecular masses can be accomplished by choosing as a calibration sequence, a representative bioagent identifying amplicon (from a specific species of bioagent) and performing, for example, a 2 to 8 nucleobase deletion or

insertion within the variable region between the two priming sites. The amplified sample containing the bioagent identifying amplicon and the calibration amplicon is then subjected to molecular mass analysis by mass spectrometry, for example. The resulting molecular mass analysis of the nucleic acid of the bioagent and of the calibration sequence provides molecular mass data and abundance data for the nucleic acid of the bioagent and of the calibration sequence. The molecular mass data obtained for the nucleic acid of the bioagent enables identification of the unknown bioagent and the abundance data enables calculation of the quantity of the bioagent, based on the knowledge of the quantity of calibration polynucleotide contacted with the sample.

[0081] In some embodiments, the identity and quantity of a particular bioagent is determined using the process illustrated in Figure 7. For instance, to a sample containing nucleic acid of an unknown bioagent are added primers (500) and a known quantity of a calibration polynucleotide (505). The total nucleic acid in the sample is subjected to an amplification reaction (510) to obtain amplification products. The molecular masses of amplification products are determined (515) from which are obtained molecular mass and abundance data. The molecular mass of the bioagent identifying amplicon (520) provides the means for its identification (525) and the molecular mass of the calibration amplicon obtained from the calibration polynucleotide (530) provides the means for its identification (535). The abundance data of the bioagent identifying amplicon is recorded (540) and the abundance data for the calibration data is recorded (545), both of which are used in a calculation (550) which determines the quantity of unknown bioagent in the sample.

[0082] In some embodiments, construction of a standard curve where the amount of calibration polynucleotide spiked into the sample is varied, provides additional resolution and improved confidence for the determination of the quantity of bioagent in the sample. The use of standard curves for analytical determination of molecular quantities is well known to one with ordinary skill and can be performed without undue experimentation.

[0083] In some embodiments, multiplex amplification is performed where multiple bioagent identifying amplicons are amplified with multiple primer pairs which also amplify the corresponding standard calibration sequences. In this or other embodiments, the standard calibration sequences are optionally included within a single vector which functions as the

calibration polynucleotide. Multiplex amplification methods are well known to those with ordinary skill and can be performed without undue experimentation.

[0084] In some embodiments, the calibrant polynucleotide is used as an internal positive control to confirm that amplification conditions and subsequent analysis steps are successful in producing a measurable amplicon. Even in the absence of copies of the genome of a bioagent, the calibration polynucleotide should give rise to a calibration amplicon. Failure to produce a measurable calibration amplicon indicates a failure of amplification or subsequent analysis step such as amplicon purification or molecular mass determination. Reaching a conclusion that such failures have occurred is in itself, a useful event.

[0085] In some embodiments, the calibration sequence is inserted into a vector which then itself functions as the calibration polynucleotide. In some embodiments, more than one calibration sequence is inserted into the vector that functions as the calibration polynucleotide. Such a calibration polynucleotide is herein termed a "combination calibration polynucleotide." The process of inserting polynucleotides into vectors is routine to those skilled in the art and can be accomplished without undue experimentation. Thus, it should be recognized that the calibration method should not be limited to the embodiments described herein. The calibration method can be applied for determination of the quantity of any bioagent identifying amplicon when an appropriate standard calibrant polynucleotide sequence is designed and used. The process of choosing an appropriate vector for insertion of a calibrant is also a routine operation that can be accomplished by one with ordinary skill without undue experimentation.

[0086] The present invention also provides kits for carrying out, for example, the methods described herein. In some embodiments, the kit may comprise a sufficient quantity of one or more primer pairs to perform an amplification reaction on a target polynucleotide from a bioagent to form a bioagent identifying amplicon. In some embodiments, the kit may comprise from one to fifty primer pairs, from one to twenty primer pairs, from one to ten primer pairs, or from two to five primer pairs. In some embodiments, the kit may comprise one or more primer pairs recited in Table 1.

[0087] In some embodiments, the kit may comprise one or more broad range survey primer(s), division wide primer(s), clade group primer(s) or drill-down primer(s), or any combination thereof. A kit may be designed so as to comprise particular primer pairs for identification of a

particular bioagent. For example, a broad range survey primer kit may be used initially to identify an unknown bioagent as a member of the *Bacillus/Clostridia* group. Another example of a division-wide kit may be used to distinguish *Bacillus anthracis*, *Bacillus cereus* and *Bacillus thuringiensis* from each other. A clade group primer kit may be used, for example, to identify an unknown bacterium as a member of the *Bacillus cereus* clade group. A drill-down kit may be used, for example, to identify genetically engineered *Bacillus anthracis*. In some embodiments, any of these kits may be combined to comprise a combination of broad range survey primers and division-wide primers, clade group primers or drill-down primers, or any combination thereof, for identification of an unknown bacterial bioagent.

[0088] In some embodiments, the kit may contain standardized calibration polynucleotides for use as internal amplification calibrants. Internal calibrants are described in commonly owned U.S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[0089] In some embodiments, the kit may also comprise a sufficient quantity of reverse transcriptase (if an RNA virus is to be identified for example), a DNA polymerase, suitable nucleoside triphosphates (including any of those described above), a DNA ligase, and/or reaction buffer, or any combination thereof, for the amplification processes described above. A kit may further include instructions pertinent for the particular embodiment of the kit, such instructions describing the primer pairs and amplification conditions for operation of the method. A kit may also comprise amplification reaction containers such as microcentrifuge tubes and the like. A kit may also comprise reagents or other materials for isolating bioagent nucleic acid or bioagent identifying amplicons from amplification, including, for example, detergents, solvents, or ion exchange resins which may be linked to magnetic beads. A kit may also comprise a table of measured or calculated molecular masses and/or base compositions of bioagents using the primer pairs of the kit.

[0090] In order that the invention disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the invention in any manner. Throughout these examples, molecular cloning reactions, and other standard recombinant DNA techniques, were carried out according to methods described in Maniatis et al., Molecular Cloning - A Laboratory Manual,

2nd ed., Cold Spring Harbor Press (1989), using commercially available reagents, except where otherwise noted.

## EXAMPLES

### [0091] Example 1: Selection of Primers That Define Bioagent Identifying Amplicons

[0092] For design of primers that define bacterial bioagent identifying amplicons, relevant sequences from, for example, GenBank are obtained, aligned and scanned for regions where pairs of PCR primers would amplify products of about 45 to about 200 nucleotides in length and distinguish species from each other by their molecular masses or base compositions. A typical process shown in Figure 2 is employed.

[0093] A database of expected base compositions for each primer region is generated using an *in silico* PCR search algorithm, such as (ePCR). An existing RNA structure search algorithm (Macke et al., Nuc. Acids Res., 2001, 29, 4724-4735, which is incorporated herein by reference in its entirety) has been modified to include PCR parameters such as hybridization conditions, mismatches, and thermodynamic calculations (SantaLucia, Proc. Natl. Acad. Sci. U.S.A., 1998, 95, 1460-1465, which is incorporated herein by reference in its entirety). This also provides information on primer specificity of the selected primer pairs.

[0094] Table 1 represents a collection of primers (sorted by forward primer name) designed to identify bacteria using the methods herein described. The forward or reverse primer name indicates the gene region of bacterial genome to which the primer hybridizes relative to a reference sequence eg: the forward primer name 16S\_EC\_1077\_1106 indicates that the primer hybridizes to residues 1077-1106 of the gene encoding 16S ribosomal RNA in an *E. coli* reference sequence represented by a sequence extraction of coordinates 4033120..4034661 from GenBank gi number 16127994 (as indicated in Table 2). As an additional example: the forward primer name BONTA\_X52066\_450\_473 indicates that the primer hybridizes to residues 450-437 of the gene encoding *Clostridium botulinum* neurotoxin type A (BoNT/A) represented by GenBank Accession No. X52066 (primer pair name codes appearing in Table 1 are defined in Table 2). In Table 1, U<sup>a</sup> = 5-propynyluracil; C<sup>a</sup> = 5-propynylcytosine; \* = phosphorothioate linkage. The primer pair number is an in-house database index number.

**Table 1: Primer Pairs for Identification of Bacterial Bioagents**

Primer pair number	For. primer name	Forward sequence	For. SEQ ID NO:	Rev. primer name	Reverse sequence	Rev. SEQ ID NO:
1	16S_EC_107	GTCAGATGTTGGGTAA	1	16S_EC_1175	GACGTCATCCCCACCTTC	368

	7 1106 F	GTCCCGTAACGAG		1195 R	TC	
266	16S_EC_108 2 1100 F	ATGTTGGGTTAAGTCCC GC	2	16S_EC_1177 _1196_10G_1 1G R	TGACGTCATGGCCACCTTC C	372
265	16S_EC_108 2 1100 F	ATGTTGGGTTAAGTCCC GC	2	16S_EC_1177 _1196_10G R	TGACGTCATGGCCACCTTC C	373
230	16S_EC_108 2 1100 F	ATGTTGGGTTAAGTCCC GC	2	16S_EC_1177 _1196 R	TGACGTCATCCCCACCTTC C	374
263	16S_EC_108 2 1100 F	ATGTTGGGTTAAGTCCC GC	2	16S_EC_1525 1541 R	AAGGAGGTGATCCAGCC	382
2	16S_EC_108 2 1106 F	ATGTTGGGTTAAGTCCC GCAACGAG	3	16S_EC_1175 1197 R	TTGACGTCATCCCCACCTT CCTC	371
278	16S_EC_109 0 1111 2 F	TTAAGTCCCGCAACGAG CGCAA	4	16S_EC_1175 _1196 R	TGACGTCATCCCCACCTTC CTC	369
361	16S_EC_109 0 1111 2_T MOD F	TTTAAGTCCCGCAACGA GCGCAA	5	16S_EC_1175 _1196_TMOD_ R	TTGACGTCATCCCCACCTT CCTC	370
3	16S_EC_109 0 1111 F	TTAAGTCCCGCAACGAT CGCAA	6	16S_EC_1175 _1196 R	TGACGTCATCCCCACCTTC CTC	369
256	16S_EC_109 2 1109 F	TAGTCCCGCAACGAGC C	7	16S_EC_1174 _1195 R	GACGTCATCCCCACCTTCC TCC	367
159	16S_EC_110 0 1116 F	CAACGAGCGCAACCCTT	8	16S_EC_1174 _1188 R	TCCCCACCTTCTCTCC	366
247	16S_EC_119 5 1213 F	CAAGTCATCATGGCCCT TA	9	16S_EC_1525 1541 R	AAGGAGGTGATCCAGCC	382
4	16S_EC_122 2 1241 F	GCTACACACGTGCTACA ATG	10	16S_EC_1303 _1323 R	CGAGTTGCAGACTGCGATC CG	376
232	16S_EC_130 3 1323 F	CGGATTGGAGTCTGCAA CTCG	11	16S_EC_1389 _1407 R	GACGGGCGGTGTGTACAAG	378
5	16S_EC_133 2 1353 F	AAGTCGGAATCGCTAGT AATCG	12	16S_EC_1389 _1407 R	GACGGGCGGTGTGTACAAG	378
252	16S_EC_136 7 1387 F	TACGGTGAATACGTTCC CGGG	13	16S_EC_1485 _1506 R	ACCTTGTACGACTTCACC CCA	379
250	16S_EC_138 7 1407 F	GCCTGTGTACACACCTCC CGTC	14	16S_EC_1494 _1513 R	CACGGCTACCTTGTGTACGA C	381
231	16S_EC_138 9 1407 F	CTTGTACACACCGCCCG TC	15	16S_EC_1525 _1541 R	AAGGAGGTGATCCAGCC	382
251	16S_EC_139 0 1411 F	TTGTACACACCGCCCGT CATAC	16	16S_EC_1486 _1505 R	CCTTGTACGACTTCACCC C	380
6	16S_EC_30_ 54 F	TGAACGCTGGTGGCATG CTTAACAC	17	16S_EC_105_ 126 R	TACGCATTACTCACCCGTC CGC	361
243	16S_EC_314 332 F	CACTGGAAGTGAGACAC GG	18	16S_EC_556_ 575 R	CTTACGCCCAGTAATTCC G	385
7	16S_EC_38_ 64 F	GTGGCATGCCTAATACZA TGCAAGTCG	19	16S_EC_101_ 120 R	TTACTCACCCGTCGCCCGC T	357
279	16S_EC_405 432 F	TGAGTGATGAAGGCCTT AGGGTTGTAAA	20	16S_EC_507_ 527 R	CGGCTGTGGCAGCAAGTT AG	384
8	16S_EC_49_ 68 F	TAACACATGCAAGTCGA ACG	21	16S_EC_104_ 120 R	TTACTCACCCGTCGCC	359
275	16S_EC_49_ 68 F	TAACACATGCAAGTCGA ACG	21	16S_EC_1061 _1078 R	ACGACACGAGCTGACGAC	364
274	16S_EC_49_ 68 F	TAACACATGCAAGTCGA ACG	21	16S_EC_880_ 894 R	CGTACTCCCCAGGCG	390
244	16S_EC_518 536 F	CCAGCAGCCCGGTAAAT AC	22	16S_EC_774_ 795 R	GTATCTAATCCTGTTTGCT CCC	387
226	16S_EC_556 575 F	CGGAATTACTGGGCGTA AAG	23	16S_EC_683_ 700 R	CGCATTTACCCGCTACAC	386
264	16S_EC_556 575 F	CGGAATTACTGGGCGTA AAG	23	16S_EC_774_ 795 R	GTATCTAATCCTGTTTGCT CCC	387
273	16S_EC_683 700 F	GTGTAGCGGTGAAATGC G	24	16S_EC_1303 _1323 R	CGAGTTGCAGACTGCGATC CG	377
9	16S_EC_683 700 F	GTGTAGCGGTGAAATGC G	24	16S_EC_774_ 795 R	GTATCTAATCCTGTTTGCT CCC	387
158	16S_EC_683 700 F	GTGTAGCGGTGAAATGC G	24	16S_EC_880_ 894 R	CGTACTCCCCAGGCG	390
245	16S_EC_683 700 F	GTGTAGCGGTGAAATGC G	24	16S_EC_967_ 985 R	GGTAAGGTTCTTCGCGTTG	396
294	16S_EC_7_3 3 F	GAGAGTTTGATCCTGGC TCAGAACGAA	25	16S_EC_101_ 122 R	TGTTACTACCCGCTGTGCC ACT	358
10	16S_EC_713 732 F	AGAACACCGATGGCGAZ GGC	26	16S_EC_789_ 809 R	CGTGGACTACCAGGGTATC TA	388
346	16S_EC_713 732_TMOD_ F	TAGAACACCGATGGCGZA AGGC	27	16S_EC_789_ 809_TMOD R	TCGTGGACTACCAGGGTAT CTA	389
228	16S_EC_774	GGGAGCAAACAGGATTZA	28	16S_EC_880	CGTACTCCCCAGGCG	390



	795 F	GATAC		894 R	
11	16S_EC_785 806 F	GGATTAGAGACCCCTGGT AGTCC	29	16S_EC_880_ 897 R	GGCCGTACTCCCCAGGCG 391
347	16S_EC_785 806_TMOD_ F	TGGATTAGAGACCCCTGG TAGTCC	30	16S_EC_880_ 897_TMOD_R	TGGCCGTACTCCCCAGGCG 392
12	16S_EC_785 810 F	GGATTAGATACCCCTGGT AGTCCACGC	31	16S_EC_880_ 897_2_R	GGCCGTACTCCCCAGGCG 391
13	16S_EC_789 810 F	TAGATACCCCTGGTAGTC CACGC	32	16S_EC_880_ 894 R	CGTACTCCCCAGGCG 390
255	16S_EC_789 810 F	TAGATACCCCTGGTAGTC CACGC	32	16S_EC_882_ 899 R	GCGACCGTACTCCCCAGG 393
254	16S_EC_791 812 F	GATACCCCTGGTAGTCCA CACCG	33	16S_EC_886_ 904 R	GCCTTGCGACCGTACTCCC 394
248	16S_EC_8_2 7 F	AGAGTTTGATCATGGCT CAG	34	16S_EC_1525 1541 R	AAGGAGGTGATCCAGCC 382
242	16S_EC_8_2 7 F	AGAGTTTGATCATGGCT CAG	34	16S_EC_342_ 358 R	ACTGCTGCCTCCCGTAG 383
253	16S_EC_804 822 F	ACCACGCCGTAAACGAT GA	35	16S_EC_909_ 929 R	CCCCCGTCAATTCCTTTGA GT 395
246	16S_EC_937 954 F	AAGCGGTGGAGCATGTG G	36	16S_EC_1220 1240 R	ATTGTAGCACGTGTGTAGC CC 375
14	16S_EC_960 981 F	TTCGATGCAACGCGAAG AACCT	37	16S_EC_1054 1073 R	ACGAGCTGACGACAGCCAT G 362
348	16S_EC_960 981_TMOD_ F	TTTCGATGCAACGCGAA GAACCT	38	16S_EC_1054 1073_TMOD_ R	TACGAGCTGACGACAGCCA TG 363
119	16S_EC_969 985_1P_F	ACGCGAAGAACCTTA U <sup>c</sup>	39	16S_EC_1061 1078_2P_R	ACGACACGAGU <sup>c</sup> C <sup>a</sup> GACGAC 364
15	16S_EC_969 985 F	ACGCGAAGAACCTTACC	39	16S_EC_1061 1078 R	ACGACACGAGCTGACGAC 364
272	16S_EC_969 985 F	ACGCGAAGAACCTTACC	40	16S_EC_1389 1407 R	GACGGGCGGTGTGTACAAG 378
344	16S_EC_971 990 F	GCGAAGAACCTTACCAG GTC	41	16S_EC_1043 1062 R	ACAACCATGCACCACCTGT C 360
120	16S_EC_972 985_2P_F	CGAAGAAU <sup>u</sup> T <sup>t</sup> TACC	42	16S_EC_1064 1075_2P_R	ACACGAGU <sup>u</sup> C <sup>a</sup> GAC 365
121	16S_EC_972 985 F	CGAAGAACCTTACC	42	16S_EC_1064 1075 R	ACACGAGCTGAC 365
1073	23S_BRM_11 10_1129_F	TGCGCGGAAGATGTAAC GGG	43	23S_BRM_117 6_1201_R	TGCGAGGCTTACAGAACGC TCTCCTA 397
1074	23S_BRM_51 5_536_F	TGCATACAAACAGTCGG AGCCT	44	23S_BRM_616 635_R	TCGGACTCGCTTTCGCTAC G 398
241	23S_BS_- 68_-44_F	AAACTAGATAACAGTAG ACATCAC	45	23S_BS_5_21 R	GTGCGCCCTTTCTAACTT 399
235	23S_EC_160 2_1620_F	TACCCCAAACCGACACA GG	46	23S_EC_1686 1703 R	CCTTCTCCCGAAGTTACG 402
236	23S_EC_168 5_1703_F	CCGTAACCTTCGGGAGAA GG	47	23S_EC_1828 1842 R	CACCGGGCAGGCGTC 403
16	23S_EC_182 6_1843_F	CTGACACCTGCCCGGTG C	48	23S_EC_1906 1924 R	GACCGTTATAGTTACGGCC 404
349	23S_EC_182 6_1843_TMO D_F	TCTGACACCTGCCCGGT GC	49	23S_EC_1906 1924_TMOD_ R	TGACCGTTATAGTTACGGC C 405
237	23S_EC_182 7_1843_F	GACGCCTGCCCGGTGC	50	23S_EC_1929 1949 R	CCGACAAGGAATTTTCGCTA CC 407
249	23S_EC_183 1_1849_F	ACCTGCCAGTGCTGGA AG	51	23S_EC_1919 1936 R	TCGCTACCTTAGGACCGT 406
234	23S_EC_187 207_F	GGGAAGTGAACATCTA AGTA	52	23S_EC_242_ 256 R	TTGCTCGCCGCTAC 408
233	23S_EC_23_ 37_F	GGTGGATGCCTTGGC	53	23S_EC_115_ 130 R	GGGTTTCCCCATTCCG 401
238	23S_EC_243 4_2456_F	AAGGTACTCCGGGGATA ACAGGC	54	23S_EC_2490 2511 R	AGCCGACATCGAGGTGCCA AAC 409
257	23S_EC_258 6_2607_F	TAGAACGTCGCGAGACA GTTTCG	55	23S_EC_2658 2677 R	AGTCCATCCCGGTCTCTC G 411
239	23S_EC_259 9_2616_F	GACAGTTCGGTCCCTAT C	56	23S_EC_2653 2669 R	CCGGTCTCTCGTACTA 410
18	23S_EC_264 5_2669_2_F	CTGTCCCTAGTACGAGA GGACCGG	57	23S_EC_2751 2767 R	GTTTCATGCTTAGATGCTT TCAGC 417
17	23S_EC_264 5_2669_F	TCTGTCCCTAGTACGAG AGGACCGG	58	23S_EC_2744 2761 R	TGCTTAGATGCTTTTCAGC 414
118	23S_EC_264 6_2667_F	CTGTCTCTAGTACGAGA GGACC	59	23S_EC_2745 2765 R	TTTCGTGCTTAGATGCTTT AG 415
360	23S_EC_264	TCTGTCTCTAGTACGAG	60	23S_EC_2745	TTTCGTGCTTAGATGCTTT 416

	6_2667_TMO D F	AGGACC		_2765_TMOD_ R	CAG	
147	23S_EC_265 2_2669_F	CTAGTACGAGAGGACCG G	61	23S_EC_2741 2760_R	ACTTAGATGCTTTCAGCGG T	413
240	23S_EC_265 3_2669_F	TAGTACGAGAGGACCGG	62	23S_EC_2737 2758_R	TTAGATGCTTTCAGCACTT ATC	412
20	23S_EC_493 518_2_F	GGGGAGTGAAGAGATC CTGAAACCG	63	23S_EC_551_ 571_2_R	ACAAAAGGCACGCCATCAC CC	418
19	23S_EC_493 518_F	GGGGAGTGAAGAGATC CTGAAACCG	63	23S_EC_551_ 571_R	ACAAAAGGTACGCCGTCAC CC	419
21	23S_EC_971 992_F	CGAGAGGGAAACAACCC AGACC	64	23S_EC_1059 1077_R	TGGCTGCTTCTAAGCCAAC	400
1158	AB_MLST- 11- OIF007_120 2_1225_F	TCGTGCCCGCAATTTGC ATAAAGC	65	AB_MLST-11- OIF007_1266 1296_R	TAATGCCGGGTAGTGCAAT CCATTCTTCTAG	420
1159	AB_MLST- 11- OIF007_120 2_1225_F	TCGTGCCCGCAATTTGC ATAAAGC	65	AB_MLST-11- OIF007_1299 1316_R	TGCACCTGCGGTCGAGCG	421
1160	AB_MLST- 11- OIF007_123 4_1264_F	TTGTAGCACAGCAAGGC AAATTTCTTGAAAC	66	AB_MLST-11- OIF007_1335 1362_R	TGCCATCCATAATCACGCC ATACTGACG	422
1161	AB_MLST- 11- OIF007_132 7_1356_F	TAGGTTTACGTCAGTAT GGCGTGATTATGG	67	AB_MLST-11- OIF007_1422 1448_R	TGCCAGTTTCCACATTTC CGTTCGTG	423
1162	AB_MLST- 11- OIF007_134 5_1369_F	TCGTGATTATGGATGGC AACGTGAA	68	AB_MLST-11- OIF007_1470 1494_R	TCGCTTGAGTGATGTCATG ATTGCG	424
1163	AB_MLST- 11- OIF007_135 1_1375_F	TTATGGATGGCAACGTG AAACGCGT	69	AB_MLST-11- OIF007_1470 1494_R	TCGCTTGAGTGATGTCATG ATTGCG	424
1164	AB_MLST- 11- OIF007_138 7_1412_F	TCTTTGCCATTGAAGAT GACTTAAGC	70	AB_MLST-11- OIF007_1470 1494_R	TCGCTTGAGTGATGTCATG ATTGCG	424
1165	AB_MLST- 11- OIF007_154 2_1569_F	TACTAGCGGTAAGCTTA AACAAGATTGC	71	AB_MLST-11- OIF007_1656 1680_R	TGAGTCGGGTTCACTTTAC CTGGCA	425
1166	AB_MLST- 11- OIF007_156 6_1593_F	TTGCCAATGATATTCGT TGTTAGCAAG	72	AB_MLST-11- OIF007_1656 1680_R	TGAGTCGGGTTCACTTTAC CTGGCA	425
1167	AB_MLST- 11- OIF007_161 1_1638_F	TCGGCGAAATCCGTATT CCTGAAAATGA	73	AB_MLST-11- OIF007_1731 1757_R	TACCGGAAGCACCAGCGAC ATTAATAG	427
1168	AB_MLST- 11- OIF007_172 6_1752_F	TACCACTATTAATGTCG CTGGTGCTTC	74	AB_MLST-11- OIF007_1790 1821_R	TGCAACTGAATAGATTGCA GTAAGTTATAAGC	428
1169	AB_MLST- 11- OIF007_179 2_1826_F	TTATACTTACTGCAAT CTATTCAGTTGCTTGGT G	75	AB_MLST-11- OIF007_1876 1909_R	TGAATTATGCAAGAAGTGA TCAATTTTCTCACGA	429
1170	AB_MLST- 11- OIF007_179 2_1826_F	TTATACTTACTGCAAT CTATTCAGTTGCTTGGT G	75	AB_MLST-11- OIF007_1895 1927_R	TGCCGTAACATAAGA GAATTATGCAAGAA	430
1152	AB_MLST- 11- OIF007_185 214_F	TATGTTTCAATGTAC AAGGTGAAGTGCG	76	AB_MLST-11- OIF007_291_ 324_R	TCACAGGTTCTACTTCATC AATAATTTCCATTGC	432
1171	AB_MLST- 11- OIF007_197 0_2002_F	TGGTTATGTACCAAATA CTTTGTCTGAAGATGG	77	AB_MLST-11- OIF007_2097 2118_R	TGACGGCATCGATACCACC GTC	431
1154	AB_MLST- 11- OIF007_206 239_F	TGAAGTGCGTGATGATA TCGATGCACTTGATGTA	78	AB_MLST-11- OIF007_318_ 344_R	TCCGCCAAAACTCCCTTT TTCACAGG	433

1153	AB_MLST-11- OIF007_260 289 F	TGGAACGTTATCAGGTG CCCCAAAATTCG	79	AB_MLST-11- OIF007_364 393 R	T* TGCAATCGACATATCCAT T* TCACCATGCC	434
1155	AB_MLST-11- OIF007_522 552 F	TCGGTTTAGTAAAGAA CGTATTGCTCAACC	80	AB_MLST-11- OIF007_587 610 R	T* TCTGCTTGAGGAATAGTG C GTGG	435
1156	AB_MLST-11- OIF007_547 571 F	TCAACCTGACTGCGTGA ATGGTTGT	81	AB_MLST-11- OIF007_656 686 R	T* ACGTTCTACGATTTCTTC A TCAGGTACATC	436
1157	AB_MLST-11- OIF007_601 627 F	TCAAGCAGAAGCTTTGG AAGAAGAAGG	82	AB_MLST-11- OIF007_710 736 R	T* ACAACGTGATAAACACGA C CAGAAGC	437
1151	AB_MLST-11- OIF007_62 91 F	TGAGATTGCTGAACATT TAATGCTGATTGA	83	AB_MLST-11- OIF007_169 203 R	T* TGTACATTGAAACAATA T* GCATGACATGTGAAT	426
1100	ASD_FRT_1_ 29 F	TTGCTTAAAGTTGGTTT TATTGGTTGGCG	84	ASD_FRT_86 116 R	T* GAGATGTCGAAAAAACG T* TGGCAAAATAC	439
1101	ASD_FRT_43 76 F	TCAGTTTAAATGTCTCG TATGATCGAATCAAAAG	85	ASD_FRT_129 156 R	T* CCATATTGTTGCATAAAA C CTGTTGGC	438
291	ASPS_EC_40 5 422 F	GCACAACCTGCGGCTGC G	86	ASPS_EC_521 538 R	A CGGCACGAGGTAGTCGC	440
485	BONTA_X520 66 450 473 F	TCTAGTAATAATAGGAC CCTCAGC	87	BONTA_X5206 6 517 539 R	T* AACCATTTCGCGTAAGAT T* CAA	441
486	BONTA_X520 66 450 473 P F	T*U* <sup>a</sup> C* <sup>a</sup> AGTAATAATAG GA*U* <sup>a</sup> U* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> AG C	87	BONTA_X5206 6 517 539P R	T* AACCA* <sup>a</sup> C* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> GC GTAAGA* <sup>a</sup> C* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> AA	441
481	BONTA_X520 66 538 552 F	TATGGCTCTACTCAA	88	BONTA_X5206 6 647 660 R	T* GTTACTGCTGGAT	443
482	BONTA_X520 66 538 552 P F	TA* <sup>a</sup> C* <sup>a</sup> GGC* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> C* <sup>a</sup> A *U* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> AA	88	BONTA_X5206 6 647 660P R	T* G* <sup>a</sup> C* <sup>a</sup> C* <sup>a</sup> A* <sup>a</sup> U* <sup>a</sup> C* <sup>a</sup> G* <sup>a</sup> U* <sup>a</sup> C * <sup>a</sup> GGAT	443
487	BONTA_X520 66 591 620 F	TGAGTCACTTGAAGTTG ATACAAATCCTCT	89	BONTA_X5206 6 644 671 R	T* CATGTGCTAATGTTACTG C TGGATCTG	442
483	BONTA_X520 66 701 720 F	GAATAGCAATTAATCCA AAT	90	BONTA_X5206 6 759 775 R	T* TACTTCTAACCCTC	444
484	BONTA_X520 66 701 720 P F	GAA* <sup>a</sup> C* <sup>a</sup> AG* <sup>a</sup> U* <sup>a</sup> AA* <sup>a</sup> C* <sup>a</sup> C *AA* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> U* <sup>a</sup> AAAT	90	BONTA_X5206 6 759 775P R	T* TA* <sup>a</sup> U* <sup>a</sup> C* <sup>a</sup> C* <sup>a</sup> U* <sup>a</sup> C* <sup>a</sup> AA* U* <sup>a</sup> U* <sup>a</sup> U* <sup>a</sup> A* <sup>a</sup> U* <sup>a</sup> C* <sup>a</sup> C	444
774	CAF1_AF053 947 33407 33430 F	TCAGTTCCGTTATCGCC ATTGCAT	91	CAF1_AF0539 47 33494 33 514 R	T* GCGGGCTGGTTCAACAAG AG	445
776	CAF1_AF053 947 33435 33457 F	TGGAACCTATTGCAACTG CTAATG	92	CAF1_AF0539 47 33499 33 517 R	T* GATGCGGGCTGGTTCAAC	446
775	CAF1_AF053 947 33515 33541 F	TCACTCTTACATATAAG GAAGGCGCTC	93	CAF1_AF0539 47 33595 33 621 R	T* CCTGTTTTATAGCCGCCA AGAGTAAG	447
777	CAF1_AF053 947 33687 33716 F	TCAGGATGGAATAACC ACCAATTCCTAC	94	CAF1_AF0539 47 33755 33 782 R	T* CAAGGTTCTCACCGTTTA CCTTAGGAG	448
22	CAPC_BA_10 4 131 F	GTTATTTAGCACTCGTT TTTAATCAGCC	95	CAPC_BA_180 205 R	T* GAATCTTGAAACACCATA C GTAACG	449
23	CAPC_BA_11 4 133 F	ACTCGTTTTAATCAGC CCG	96	CAPC_BA_185 205 R	T* GAATCTTGAAACACCATA C G	450
24	CAPC_BA_27 4 303 F	GATTATTGTTATCCTGT TATGCCATTGAG	97	CAPC_BA_349 376 R	GTAACCCCTGTCTTTGAAT T* GTATTGC	451
350	CAPC_BA_27 4 303_TMOD F	TGATTATTGTTATCCTG TTATGCCATTGAG	98	CAPC_BA_349 376_TMOD R	T* GTAACCCCTGTCTTTGAA T* GTATTGC	452
25	CAPC_BA_27 6 296 F	TTATTGTTATCCTGTTA TGCC	99	CAPC_BA_358 377 R	GGTAACCCCTGTCTTTGAA T	453
26	CAPC_BA_28 1 301 F	GTTATCCTGTTATGCCA TTTG	100	CAPC_BA_361 378 R	T* GGTAACCCCTGTCTTTG	454
27	CAPC_BA_31 5 334 F	CCGTGGTATTGGAGTTA TTG	101	CAPC_BA_361 378 R	T* GGTAACCCCTGTCTTTG	454
1053	CJST_CJ_10	TTGAGGGTATGCACCGT	102	CJST_CJ_116	T* CCCCTCATGTTTAAATGA	456

	80 1110 F	CTTTTGGATTCTTT		6 1198 R	TCAGGATAAAAAGC	
1063	CJST_CJ_12 68 1299 F	AGTTATAAACACGGCTT TCCTATGGCTTATCC	103	CJST_CJ_134 9 1379 R	TCGGTTTAAGCTCTAC ATG ATCGTAAGGATA	457
1050	CJST_CJ_12 90 1320 F	TGGCTTATCCAAATTTA GATCGTGGTTTTAC	104	CJST_CJ_140 6 1433 R	TTTGCTCATGATCTGC ATG AAGCATAAA	458
1058	CJST_CJ_16 43 1670 F	TTATCGTTTGTGGAGCT AGTGCTTATGC	105	CJST_CJ_172 4 1752 R	TGCAATGTGTGCTATG TCA GCAAAAAGAT	459
1045	CJST_CJ_16 68 1700 F	TGCTCGAGTGATTGACT TTGCTAAATTTAGAGA	106	CJST_CJ_177 4 1799 R	TGAGCGTGTGGAAAAG GAC TTGGATG	460
1064	CJST_CJ_16 80 1713 F	TGATTTTGCTAAATTTA GAGAAATGCGGATGAA	107	CJST_CJ_179 5 1822 R	TATGTGTAGTTGAGCT TAC TACATGAGC	461
1056	CJST_CJ_18 80 1910 F	TCCCAATTAATTTCTGCC ATTTTCCAGGTAT	108	CJST_CJ_198 1 2011 R	TGGTCTTACTTGCTT TGC ATAAACTTTCCA	462
1054	CJST_CJ_20 60 2090 F	TCCCGGACTTAATATCA ATGAAAATTTGTGGA	109	CJST_CJ_214 8 2174 R	TCGATCCGCATCACCA TCA AAGCAAA	463
1059	CJST_CJ_21 65 2194 F	TGCGGATCGTTTGGTGG TTGTAGATGAAAA	110	CJST_CJ_224 7 2278 R	TCCACACTGGATTGTA ATT TACCTTGTCTCTT	464
1046	CJST_CJ_21 71 2197 F	TCGTTTGGTGGTGGTAG ATGAAAAAGG	111	CJST_CJ_228 3 2313 R	TCCTTTTCAAAGCACC ATT GCTCATTATAGT	465
1057	CJST_CJ_21 85 2212 F	TAGATGAAAAGGGCGAA GTGGCTAATGG	112	CJST_CJ_228 3 2316 R	TGAATTCCTTTCAAAGC ACC ATTGCTCATTATAGT	466
1049	CJST_CJ_26 36 2668 F	TGCCTAGAAGATCTTAA AAATTTCCGCCAACTT	113	CJST_CJ_275 3 2777 R	TTGCTGCCATAGCAAA GCC TACAGC	467
1062	CJST_CJ_26 78 2703 F	TCCCAGGACACCCCTGA AATTTCAAC	114	CJST_CJ_276 0 2787 R	TGTGCTTTTTTTGCTG CCA TAGCAAAGC	468
1065	CJST_CJ_28 57 2887 F	TGGCATTCTTTATGAAG CTTGTCTTTAGCA	115	CJST_CJ_296 5 2998 R	TGCTTCAAACGCATT TTT ACATTTTCGTTAAAG	469
1055	CJST_CJ_28 69 2895 F	TGAAGCTTGTCTTTTAG CAGGACTTCA	116	CJST_CJ_297 9 3007 R	TCCTCCTGTGCTCTCA AAA CGCATTTTTA	470
1051	CJST_CJ_32 67 3293 F	TTTGATTTTACGCCGTC CTCCAGGTCG	117	CJST_CJ_335 6 3385 R	TCAAAGAACC CGCACC TAA TTCATCATTTA	471
1061	CJST_CJ_36 0 393 F	TCCTGTTATCCCTGAAG TAGTTAATCAAGTTTGT	118	CJST_CJ_443 477 R	TACAACTGGTTCAAAA ACA TTAAGCTGTAATTGTC	473
1048	CJST_CJ_36 0 394 F	TCCTGTTATCCCTGAAG TAGTTAATCAAGTTTGT T	119	CJST_CJ_442 476 R	TCAACTGGTTCAAAA CAT TAAGTTGTAATTGTCC	472
1052	CJST_CJ_5_ 39 F	TAGGCGAAGATATACAA AGAGTATTAGAAGCTAG A	120	CJST_CJ_104 137 R	TCCCTTATTTTTCTTT CTA CTACCTTCGGATAAT	455
1047	CJST_CJ_58 4 616 F	TCCAGGACAAATGTATG AAAAATGTCCAAGAAG	121	CJST_CJ_663 692 R	TTCAATTTCTGGTCCA AAG TAAGCAGTATC	474
1060	CJST_CJ_59 9 632 F	TGAAAATGTCCAAGAA GCATAGCAAAAAAGCA	122	CJST_CJ_711 743 R	TCCCGAACAATGAGTT GTA TCAACTATTTTAC	475
1096	CTXA_VBC_1 17 142 F	TCTTATGCCAAGAGGAC AGAGTGAGT	123	CTXA_VBC_19 4 218 R	TGCCTAACAAATCCCG TCT GAGTTC	476
1097	CTXA_VBC_3 51 377 F	TGTATTAGGGGCATACA GTCCTCATCC	124	CTXA_VBC_44 1 466 R	TGTCATCAAGCACC CC AAA ATGAAC	477
28	CYA_BA_105 5 1072 F	GAAAGAGTTCCGATTGG G	125	CYA_BA_1112 1130 R	TGTTGACCATGCTTCT TAG CAC	479
277	CYA_BA_134 9 1370 F	ACAACGAAGTACAATAC AAGAC	126	CYA_BA_1426 1447 R	CTTCTACATTTT TAGC CAT CAC	480
30	CYA_BA_135 3 1379 F	CGAAGTACAATACAAGA CAAAAGAAGG	127	CYA_BA_1448 1467 R	TGTTAACGGCTTCAAG ACC C	482
351	CYA_BA_135 3_1379_TMO D F	TCGAAGTACAATACAAG ACAAAAGAAGG	128	CYA_BA_1448 1467_TMOD_ R	TTGTTAACGGCTTCAA GAC CC	483
31	CYA_BA_135 9 1379 F	ACAATACAAGCAAAAAG AAGG	129	CYA_BA_1447 1461 R	CGGCTTCAAGACCCC	481
32	CYA_BA_914 937 F	CAGGTTTAGTACCAGAA CATGCAG	130	CYA_BA_999_ 1026 R	ACCACTTTTAATAAGG TTT GTAGCTAAC	484
33	CYA_BA_916 935 F	GGTTTAGTACCAGAACA TGC	131	CYA_BA_1003 1025 R	CCACTTTTAATAAGGT TTG TAGC	478
115	DNAK_EC_42 8 449 F	CGGCGTACTTCAACGAC AGCCA	132	DNAK_EC_503 522 R	CGCGGTCGGCTCGTTG ATG A	485
1102	GALE_FRT_1 68 199 F	TTATCAGTAGACCTTT TAGGTAAGCTAAGC	133	GALE_FRT_24 1 269 R	TCACCTACAGCTTTAA AGC CAGCAAAATG	486
1104	GALE_FRT_3 08 339 F	TCCAAGGTACACTAAAC TACTTGAGCTAATG	134	GALE_FRT_39 0 422 R	TCTTCTGTAAAGGGTG GTT TATTATTTCATCCCA	487
1103	GALE_FRT_8 34 865 F	TCAAAAAGCCCTAGGTA AAGAGATTCATATC	135	GALE_FRT_90 1 925 R	TAGCCTTGGCAACATC AGC AAAAC	488
1092	GLTA_RKP_1 023 1055 F	TCCGTTCTTACAAATAG CAATAGAACTTGAAGC	136	GLTA_RKP_11 29 1156 R	TTGGCGACGGTATACC CAT AGCTTTATA	489
1093	GLTA_RKP_1 043_1072_2 F	TGGAGCTTGAAGCTATC GCTCTTAAAGATG	137	GLTA_RKP_11 38 1162 R	TGAACATTTGGACGG TAT ACCCAT	490

1094	GLTA_RKP_1 043_1072_3 F	TGGAACTTGAAGCTCTC GCTCTTAAAGATG	138	GLTA_RKP_11 38_1164_R	TGTGAACATTTCGACGGT ATACCCAT	492
1090	GLTA_RKP_1 043_1072_F	TGGGACTTGAAGCTATC GCTCTTAAAGATG	139	GLTA_RKP_11 38_1162_R	TGAACATTTCGACGGTAT ACCCAT	491
1091	GLTA_RKP_4 00_428_F	TCTTCTCATCCTATGGC TATTATGCTTGC	140	GLTA_RKP_49 9_529_R	TGGTGGGTATCTTAGCAAT CATTCTAATAGC	493
1095	GLTA_RKP_4 00_428_F	TCTTCTCATCCTATGGC TATTATGCTTGC	140	GLTA_RKP_50 5_534_R	TGCGATGGTAGGTATCTTA GCAATCATTCT	494
224	GROL_EC_21 9_242_F	GGTGAAAGAAGTTGCCT CTAAAGC	141	GROL_EC_328 350_R	TTCAGGTCCATCGGTTCA TGCC	496
280	GROL_EC_49 6_518_F	ATGGACAAGGTTGGCAA GGAAGC	142	GROL_EC_577 596_R	TAGCCGCGTTCGAATTGCA T	498
281	GROL_EC_51 1_536_F	AAGGAAGGCGTGATCAC CGTTGAAGA	143	GROL_EC_571 593_R	CCGCGGTCGAATTGCATGC CTTC	497
220	GROL_EC_94 1_959_F	TGGAAGATCTGGGTGAG GC	144	GROL_EC_103 9_1060_R	CAATCTGCTGACGGATCTG AGC	495
924	GYRA_AF100 557_4_23_F	TCTGCCCCGTGTCGTTGG TGA	145	GYRA_AF1005 57_119_142_ R	TCGAACCGAAGTTACCCCTG ACCAT	499
925	GYRA_AF100 557_70_94_ F	TCCATTGTTTCGTATGGC TCAAGACT	146	GYRA_AF1005 57_178_201_ R	TGCCAGCTTAGTCATACGG ACTTC	500
926	GYRB_AB008 700_19_40_ F	TCAGGTGGCTTACACGG CGTAG	147	GYRB_AB0087 00_111_140_ R	TATTGCGGATCACCATGAT GATATTCTTGC	501
927	GYRB_AB008 700_265_29 2_F	TCTTCTTGAATGCTGG TGTACGTATCG	148	GYRB_AB0087 00_369_395_ R	TCGTTGAGATGGTTTTTAC CTTCGTIG	502
928	GYRB_AB008 700_368_39 4_F	TCAACGAAGGTAAAAAC CATCTCAACG	149	GYRB_AB0087 00_466_494_ R	TTTGTGAAACAGCGAACAT TTTCTTGGTA	503
929	GYRB_AB008 700_477_50 4_F	TGTTTCGCTGTTTCACAA ACAACATTCCA	150	GYRB_AB0087 00_611_632_ R	TCACGCGCATCATCACCAG TCA	504
949	GYRB_AB008 700_760_78 7_F	TACTTACTTGAGAATCC ACAAGCTGCAA	151	GYRB_AB0087 00_862_888_ 2_R	TCCTGCAATATCTAATGCA CTCTTACG	505
930	GYRB_AB008 700_760_78 7_F	TACTTACTTGAGAATCC ACAAGCTGCAA	151	GYRB_AB0087 00_862_888_ R	ACCTGCAATATCTAATGCA CTCTTACG	506
222	HFLB_EC_10 82_1102_F	TGGCGAACCTGGTGAAC GAAGC	152	HFLB_EC_114 4_1168_R	CTTTCGCTTTCTCGAACTC AACCAT	507
1128	HUPB_CJ_11 3_134_F	TAGTTGCTCAAACAGCT GGGCT	153	HUPB_CJ_157 188_R	TCCCTAATAGTAGAAATAA CTGCATCAGTAGC	509
1130	HUPB_CJ_76 102_F	TCCCGGAGCTTTTATGA CTAAAGCAGAT	154	HUPB_CJ_114 135_R	TAGCCGAGCTGTTTGAGCA ACT	508
1129	HUPB_CJ_76 102_F	TCCCGGAGCTTTTATGA CTAAAGCAGAT	154	HUPB_CJ_157 188_R	TCCCTAATAGTAGAAATAA CTGCATCAGTAGC	510
1079	ICD_CXB_17 6_198_F	TCGCCGTGGAAAAATCC TACGCT	155	ICD_CXB_224 247_R	TAGCCTTTTCTCCGGCGTA GATCT	512
1078	ICD_CXB_92 120_F	TTCTGACCGACCCATT ATTCCCTTTATC	156	ICD_CXB_172 194_R	TAGGATTTTTCACGGCGG CATC	510
1077	ICD_CXB_93 120_F	TCCTGACCGACCCATTA TTCCCTTTATC	157	ICD_CXB_172 194_R	TAGGATTTTTCACGGCGG CATC	511
221	INFB_EC_11 03_1124_F	GTCGTGAAAACGAGCTG GAAGA	158	INFB_EC_117 4_1191_R	CATGATGGTCACAACCGG	513
964	INFB_EC_13 47_1367_F	TGCGTTTACCGCAATGC GTGC	159	INFB_EC_141 4_1432_R	TCGGCATCACGCCGTCGTC	514
34	INFB_EC_13 65_1393_F	TGCTCGTGGTGCACAAG TAACGGATATTA	160	INFB_EC_143 9_1467_R	TGCTGCTTTTCGCATGGTTA ATTGCTTCAA	515
352	INFB_EC_13 65_1393_TM OD_F	TTGCTCGTGGTGCACAA GTAACGGATATTA	161	INFB_EC_143 9_1467_TMOD R	TTGCTGCTTTTCGCATGGTT AATGCTTCAA	516
223	INFB_EC_19 69_1994_F	CGTCAGGGTAAATCCG TGAAGTTAA	162	INFB_EC_203 8_2058_R	AACTTCGCCTTCGGTCATG TT	517
781	INV_U22457 _1558_1581 F	TGGTAACAGAGCCTTAT AGGCGCA	163	INV_U22457 1619_1643_R	TTGCGTTGCAGATTATCTT TACCAA	518
778	INV_U22457 515_539_F	TGGCTCCTTGGTATGAC TCTGCTTC	164	INV_U22457 571_598_R	TGTTAAGTGTGTTGCGGCT GTCTTTATT	519
779	INV_U22457 699_724_F	TGCTGAGGCTTGACCG ATTATTTAC	165	INV_U22457 753_776_R	TCACGCGACGAGTGCCATC CATTTG	520
780	INV_U22457 834_858_F	TTATTTACCTGCACTCC CACAACTG	166	INV_U22457 942_966_R	TGACCCAAAGCTGAAAGCT TTACTG	521

1106	IPAH_SGF_1 13_134_F	TCCTTGACCGCCTTTCC GATAC	167	IPAH_SGF_17 2_191_R	TTTCCAGCCATGCAGCGA C	522
1105	IPAH_SGF_2 58_277_F	TGAGGACCGTGTGCGGC TCA	168	IPAH_SGF_30 1_327_R	TCCTTCTGATGCCTGATGG ACCAGGAG	523
1107	IPAH_SGF_4 62_486_F	TCAGACCATGCTCGCAG AGAACTT	169	IPAH_SGF_52 2_540_R	TGTCACCTCCCACACGCCA	524
1080	IS1111A_NC 002971_686 6_6891_F	TCAGTATGTATCCACCG TAGCCAGTC	170	IS1111A_NC0 02971_6928_ 6954_R	TAAACGTCCGATACCAATG GTTGCTC	525
1081	IS1111A_NC 002971_745 6_7483_F	TGGGTGACATTTCATCAA TTTCATCGTTC	171	IS1111A_NC0 02971_7529_ 7554_R	TCAACAACACCTCCTTATT CCCACTC	526
35	LEF_BA_103 3_1052_F	TCAAGAAGAAAAAGAGC	172	LEF_BA_1119 1135_R	GAATATCAATTTGTAGC	527
36	LEF_BA_103 6_1066_F	CAAGAAGAAAAAGAGCT TCTAAAAAGAATAC	173	LEF_BA_1119 1149_R	AGATAAGAATCACGAATA TCAATTTGTAGC	528
37	LEF_BA_756 781_F	AGCTTTTGCATATTATA TCGAGCCAC	174	LEF_BA_843_ 872_R	TCTTCCAAGGATAGATTTA TTTCTTGTTCG	530
353	LEF_BA_756 781_TMOD_ F	TAGCTTTTGCATATTAT ATCGAGCCAC	175	LEF_BA_843_ 872_TMOD_R	TTCTTCCAAGGATAGATTT ATTCTTGTTCG	531
38	LEF_BA_758 778_F	CTTTTGCATATTATATC GAGC	176	LEF_BA_843_ 865_R	AGGATAGATTTATTCTTG TTCG	529
39	LEF_BA_795 813_F	TTTACAGCTTTATGCAC CG	177	LEF_BA_883_ 900_R	TCTTGACAGCATCCGTTG	532
40	LEF_BA_883 899_F	CAACGGATGCTGGCAAG	178	LEF_BA_939_ 958_R	CAGATAAAGAATCGCTCCA G	533
782	LL_NC00314 3_2366996_ 2367019_F	TGTAGCCGCTAAGCACT ACCATCC	179	LL_NC003143 2367073_23 67097_R	TCTCATCCCGATATTACCG CCATGA	534
783	LL_NC00314 3_2367172_ 2367194_F	TGGACGGCATCACGATT CTCTAC	180	LL_NC003143 2367249_23 67271_R	TGGCAACAGCTCAACACCT TTGG	535
878	MECA_Y1405 1_3645_367 0_F	TGAAGTAGAAATGACTG AACGTCCGA	181	MECA_Y14051 3690_3719_ R	TGATCCTGAATGTTTATAT CTTTAACGCCT	536
877	MECA_Y1405 1_3774_380 2_F	TAAACAAACTACGGTA ACAATTGATCGCA	182	MECA_Y14051 3828_3854_ R	TCCCAATCTAACTTCCACA TACCATCT	537
879	MECA_Y1405 1_4507_453 0_F	TCAGGTACTGCTATCCA CCCTCAA	183	MECA_Y14051 4555_4581_ R	TGGATAGACGTCATATGAA GGTGTGCT	538
880	MECA_Y1405 1_4510_453 0_F	TGTACTGCTATCCACCC TCAA	184	MECA_Y14051 4586_4610_ R	TATTCTTCTGTACTCATGC CATACA	539
882	MECA_Y1405 1_4520_453 0_F	TU <sup>3</sup> U <sup>3</sup> AU <sup>3</sup> U <sup>3</sup> C <sup>3</sup> U <sup>3</sup> AA	185	MECA_Y14051 4590_4600P R	C <sup>3</sup> AU <sup>3</sup> C <sup>3</sup> U <sup>3</sup> AC <sup>3</sup> GU <sup>3</sup> U <sup>3</sup> A	540
883	MECA_Y1405 1_4520_453 0_F	TU <sup>3</sup> U <sup>3</sup> AU <sup>3</sup> U <sup>3</sup> C <sup>3</sup> U <sup>3</sup> AA	185	MECA_Y14051 4600_4610P R	C <sup>3</sup> AC <sup>3</sup> C <sup>3</sup> U <sup>3</sup> C <sup>3</sup> U <sup>3</sup> GC <sup>3</sup> T	541
881	MECA_Y1405 1_4669_469 8_F	TCACCAGGTTCAACTCA AAAAATATTAACA	186	MECA_Y14051 4765_4793_ R	TAACCACCCCAAGATTTAT CTTTTGGCCA	542
876	MECA_Y140 51_3315_33 41_F	TTACACATATCGTGAGC AATGAACTGA	187	MECA_Y1405 1_3367_3393 R	TGTGATATGGAGGTGTAGA AGGTGTTA	543
914	OMPA_AY485 227_272_30 1_F	TTACTCCATTATTGCTT GGTTACACTTTCC	188	OMPA_AY4852 27_364_388_ R	GAGCTGCGCCAACGAATAA ATCGTC	544
916	OMPA_AY485 227_311_33 5_F	TACACAACAATGGCGGT AAAGATGG	189	OMPA_AY4852 27_424_453_ R	TACGTCGCCTTTAACTTGG TTATATTCAGC	545
915	OMPA_AY485 227_379_40 1_F	TGCGCAGCTCTTGGTAT CGAGTT	190	OMPA_AY4852 27_492_519_ R	TGCCGTAACATAGAAGTTA CCGTGATT	546
917	OMPA_AY485 227_415_44 1_F	TGCCTCGAAGCTGAATA TAACCAAGTT	191	OMPA_AY4852 27_514_546_ R	TCGGGCGTAGTTTTTAGTA ATTAAATCAGAAGT	547
918	OMPA_AY485 227_494_52 0_F	TCAACGGTAACTTCTAT GTTACTTCTG	192	OMPA_AY4852 27_569_596_ R	TCGTGCTATTTATAGTGAC CAGCACCTA	548
919	OMPA_AY485 227_551_57 7_F	TCAAGCCGTACGTATTA TTAGGTGCTG	193	OMPA_AY4852 27_658_680_ R	TTTAAGCGCCAGAAAGCAC CAAC	550

920	OMPA AY485 227_555_58 1 F	TCCGTACGTATTATTAG GTGCTGGTCA	194	OMPA AY4852 27_635_662_ R	TCAACACCAGCGTTACCTA AAGTACCTT	549
921	OMPA AY485 227_556_58 3 F	TCGTACGTATTATTAGG TGCTGGTCACT	195	OMPA AY4852 27_659_683_ R	TCGTTTAAGCGCCAGAAAG CACCAA	551
922	OMPA AY485 227_657_67 9 F	TGTTGGTGCTTTCTGGC GCTTAA	196	OMPA AY4852 27_739_765_ R	TAAGCCAGCAAGAGCTGTA TAGTTCCA	552
923	OMPA AY485 227_660_68 3 F	TGGTGCTTTCTGGCGCT TAAACGA	197	OMPA AY4852 27_786_807_ R	TACAGGAGCAGCAGGCTTC AAG	553
1088	OMP_B_RKP_1 192_1221 F	TCTACTGATTTTGGTAA TCTTGCAGCACAG	198	OMP_B_RKP_12 88_1315 R	TAGCAGCAAAAGTTATCAC ACCTGCAGT	554
1089	OMP_B_RKP_3 417_3440 F	TGCAAGTGGTACTTCAA CATGGGG	199	OMP_B_RKP_35 20_3550 R	TGGTTGTAGTTCTCTAGT TGTTGCATTAAC	555
1087	OMP_B_RKP_8 60_890 F	TTACAGGAAGTTTAGGT GGTAATCTAAAAGG	200	OMP_B_RKP_97 2_996 R	TCCTGCAGCTCTACCTGCT CCATTA	556
41	PAG_BA_122 142 F	CAGAATCAAGTTCCCAG GGG	201	PAG_BA_190_ 209_R	CCTGTAGTAGAAGAGGTAA C	558
42	PAG_BA_123 145 F	AGAATCAAGTTCCCAGG GGTTAC	202	PAG_BA_187_ 210_R	CCCTGTAGTAGAAGAGGTAA ACCAC	557
43	PAG_BA_269 287 F	AATCTGCTATTTGGTCA GG	203	PAG_BA_326_ 344_R	TGATTATCAGCGGAAGTAG	559
44	PAG_BA_655 675 F	GAAGGATATACGGTTGA TGTC	204	PAG_BA_755_ 772_R	CCGTGCTCCATTTTTCAG	560
45	PAG_BA_753 772 F	TCCTGAAAAATGGAGCA CGG	205	PAG_BA_849_ 868_R	TCGGATAAGCTGCCACAAG G	561
46	PAG_BA_763 781 F	TGGAGCACGGCTTCTGA TC	206	PAG_BA_849_ 868_R	TCGGATAAGCTGCCACAAG G	562
912	PARC_X9581 9_123_147_ F	GGCTCAGCCATTAGTT ACCGCTAT	207	PARC_X95819 232_260 R	TCGCTCAGCAATAATTCAC TATAAGCCGA	566
913	PARC_X9581 9_43_63 F	TCAGCGCGTACAGTGGG TGAT	208	PARC_X95819 143_170 R	TTCCCTGACCTTCGATTA AAGGATAGC	563
911	PARC_X9581 9_87_110 F	TGGTGACTCGGCATGTT ATGAAGC	209	PARC_X95819 192_219 R	GGTATAACGCATCGCAGCA AAAGATTTA	564
910	PARC_X9581 9_87_110 F	TGGTGACTCGGCATGTT ATGAAGC	209	PARC_X95819 201_222 R	TTCCGTATAACGCATCGCA GCA	565
773	PLA_AF0539 45_7186_72 11 F	TTATACCGGAAACTTCC CGAAAGGAG	210	PLA_AF05394 5_7257_7280 R	TAATGCGATACTGGCCTGC AAGTC	567
770	PLA_AF0539 45_7377_74 02 F	TGACATCCGGCTCACGT TATTATGGT	211	PLA_AF05394 5_7434_7462 R	TGTAAATCCGCAAGACT TTGGCATTAG	568
771	PLA_AF0539 45_7382_74 04 F	TCCGGCTCACGTTATTA TGGTAC	212	PLA_AF05394 5_7482_7502 R	TGGTCTGAGTACCTCCTTT GC	569
772	PLA_AF0539 45_7481_75 03 F	TGCAAAGGAGGTACTCA GACCAT	213	PLA_AF05394 5_7539_7562 R	TATTGGAAATACCGGCAGC ATCTC	570
909	RECA_AF251 469_169_19 0 F	TGACATGCTTGTCGGTT CAGGC	214	RECA_AF2514 69_277_300_ R	TGGCTCATTAAGACGCGCTT GTAGA	572
908	RECA_AF251 469_43_68_ F	TGGTACATGTGCCTTCA TTGATGCTG	215	RECA_AF2514 69_140_163_ R	TTCAAGTGCTTGCTCACCA TTGTC	571
1072	RNASEP_BDP 574_592 F	TGGCACGGCCATCTCCG TG	216	RNASEP_BDP 616_635 R	TCGTTTACCCTGTCTATGC CG	573
1070	RNASEP_BKM 580_599 F	TCCGGCTAGGGAGCTTG AGC	217	RNASEP_BKM 665_686 R	TCCGATAAGCCGATCTG TGC	574
1071	RNASEP_BKM 616_637 F	TCCTAGAGGAATGGCTG CCACG	218	RNASEP_BKM 665_687 R	TGCCGATAAGCCGGATTCT GTGC	575
1112	RNASEP_BRM 325_347 F	TACCCAGGGAAAGTGC CACAGA	219	RNASEP_BRM 402_428 R	TCTCTTACCCACCCTTTC ACCCTTAC	576
1172	RNASEP_BRM 461_488 F	TAAACCCCATCGGGAGC AAGACCGAATA	220	RNASEP_BRM 542_561_2 R	TGCCTCGTGCAACCCACCC G	577
1111	RNASEP_BRM 461_488 F	TAAACCCCATCGGGAGC AAGACCGAATA	220	RNASEP_BRM 542_561 R	TGCCTCGGCAACCTACCC G	578
258	RNASEP_BS_ 43_61 F	GAGGAAAGTCCATGCTC GC	221	RNASEP_BS_3 63_384 R	GTAAGCCATGTTTGTTC ATC	579
259	RNASEP_BS_ 43_61 F	GAGGAAAGTCCATGCTC GC	221	RNASEP_BS_3 63_384 R	GTAAGCCATGTTTGTTC ATC	578
258	RNASEP_BS_ 43_61 F	GAGGAAAGTCCATGCTC GC	221	RNASEP_EC_3 45_362 R	ATAAGCCGGTCTGTGCG	581

258	RNASEP_BS_43 61 F	GAGGAAAGTCCATGCTC GC	221	RNASEP_SA_3 58 379 R	ATAAGCCATGTTCTGTTC ATC	584
1076	RNASEP_CLB 459 487 F	TAAGGATAGTGCAACAG AGATATACCGCC	222	RNASEP_CLB 498 522 R	TTTACCTCGCCTTTCCACC CTTACC	579
1075	RNASEP_CLB 459 487 F	TAAGGATAGTGCAACAG AGATATACCGCC	222	RNASEP_CLB 498 526 R	TGCTCTTACCTCACCGTTC CACCCTTACC	580
258	RNASEP_EC_61 77 F	GAGGAAAGTCCGGGCTC	223	RNASEP_BS_3 63 384 R	GTAAGCCATGTTTTGTTC ATC	578
258	RNASEP_EC_61 77 F	GAGGAAAGTCCGGGCTC	223	RNASEP_EC_3 45 362 R	ATAAGCCGGGTCTGTCTG	581
260	RNASEP_EC_61 77 F	GAGGAAAGTCCGGGCTC	223	RNASEP_EC_3 45 362 R	ATAAGCCGGGTCTGTCTG	581
258	RNASEP_EC_61 77 F	GAGGAAAGTCCGGGCTC	223	RNASEP_SA_3 58 379 R	ATAAGCCATGTTCTGTTC ATC	584
1085	RNASEP_RKP 264 287 F	TCTAAATGGTCGTGCAG TTGCGTG	224	RNASEP_RKP 295 321 R	TCTATAGAGTCCGGACTTT CCTCGTGA	582
1082	RNASEP_RKP 419 448 F	TGGTAAGAGCGCACCGG TAAGTTGGTAACA	225	RNASEP_RKP 542 565 R	TCAAGCGATCTACCCGCAT TACAA	583
1083	RNASEP_RKP 422 443 F	TAAGAGCGCACCGGTAA GTTGG	226	RNASEP_RKP 542 565 R	TCAAGCGATCTACCCGCAT TACAA	583
1086	RNASEP_RKP 426 448 F	TGCATACCGGTAAGTTG GCAACA	227	RNASEP_RKP 542 565 R	TCAAGCGATCTACCCGCAT TACAA	583
1084	RNASEP_RKP 466 491 F	TCCACCAAGAGCAAGAT CAAATAGGC	228	RNASEP_RKP 542 565 R	TCAAGCGATCTACCCGCAT TACAA	583
258	RNASEP_SA_31 49 F	GAGGAAAGTCCATGCTC AC	229	RNASEP_BS_3 63 384 R	GTAAGCCATGTTTTGTTC ATC	578
258	RNASEP_SA_31 49 F	GAGGAAAGTCCATGCTC AC	229	RNASEP_EC_3 45 362 R	ATAAGCCGGGTCTGTCTG	581
258	RNASEP_SA_31 49 F	GAGGAAAGTCCATGCTC AC	229	RNASEP_SA_3 58 379 R	ATAAGCCATGTTCTGTTC ATC	584
262	RNASEP_SA_31 49 F	GAGGAAAGTCCATGCTC AC	229	RNASEP_SA_3 58 379 R	ATAAGCCATGTTCTGTTC ATC	584
1098	RNASEP_VBC 331 349 F	TCCGCGGAGTTGACTGG GT	230	RNASEP_VBC 388 414 R	TGACTTTCTCCCCCTTAT CAGTCTCC	585
66	RPLB_EC_65 0 679 F	GACCTACAGTAAGAGGT TCTGTAATGAACC	231	RPLB_EC_739 762 R	TCCAAGTGCTGGTTTACCC CATGG	591
356	RPLB_EC_65 0 679 TMOD F	TGACCTACAGTAAGAGG TTCTGTAATGAACC	232	RPLB_EC_739 762 TMOD R	TTCCAAGTGCTGGTTTACC CCATGG	592
73	RPLB_EC_66 9 698 F	TGTAATGAACCCTAATG ACCATCCACACGG	233	RPLB_EC_735 761 R	CCAAGTGCTGGTTTACCCC ATGGAGTA	586
74	RPLB_EC_67 1 700 F	TAATGAACCCTAATGAC CATCCACACGGTG	234	RPLB_EC_737 762 R	TCCAAGTGCTGGTTTACCC CATGGAG	590
67	RPLB_EC_68 8 710 F	CATCCACACGGTGGTGG TGAAGG	235	RPLB_EC_736 757 R	GTGCTGGTTTACCCCATGG AGT	587
70	RPLB_EC_68 8 710 F	CATCCACACGGTGGTGG TGAAGG	235	RPLB_EC_743 771 R	TGTTTTGTATCCAAGTGCT GGTTTACCCC	593
357	RPLB_EC_68 8 710 TMOD F	TCATCCACACGGTGGTG GTGAAGG	236	RPLB_EC_736 757 TMOD R	TGTGCTGGTTTACCCCATG GAGT	588
449	RPLB_EC_69 0 710 F	TCCACACGGTGGTGGTG AAGG	237	RPLB_EC_737 758 R	TGTGCTGGTTTACCCCATG GAG	589
113	RPOB_EC_13 36 1353 F	GACCACCTCGGCAACCG T	238	RPOB_EC_143 8 1455 R	TTGCTCTCGGCTTGCC	594
963	RPOB_EC_15 27 1549 F	TCAGCTGTGCGAGTTCA TGGACC	239	RPOB_EC_163 0 1649 R	TCGTCGCGGACTTCGAAGC C	595
72	RPOB_EC_18 45 1866 F	TATCGCTCAGGCGAACT CCAAC	240	RPOB_EC_190 9 1929 R	GCTGGATTGCGCTTTGCTA CG	596
359	RPOB_EC_18 45 1866 TMOD F	TTATCGCTCAGGCGAAC TCCAAC	241	RPOB_EC_190 9 1929 TMOD R	TGCTGGATTGCGCTTTGCT ACG	597
962	RPOB_EC_20 05 2027 F	TCGTTCCCTGGAACACGA TGACGC	242	RPOB_EC_204 1 2064 R	TTGACGTTGCATGTTTCGAG CCCAT	598
69	RPOB_EC_37 62 3790 F	TCAACAACCTCTTGGAG GTAAAGCTCAGT	243	RPOB_EC_383 6 3865 R	TTTCTTGAAGAGTATGAGC TGCTCCGTAAG	600
111	RPOB_EC_37 75 3803 F	CTTGGAGGTAAGTCTCA TTTTGGTGGGCA	244	RPOB_EC_382 9 3858 R	CGTATAAGCTGCACCATAA GCTTGTAATGC	599
940	RPOB_EC_37 98 3821 F	TGGGCAGCGTTTCGGCG AAATGGA	245	RPOB_EC_386 2 3889 2 R	TGTCCGACTTGACGGTTAG CATTCCTG	604
939	RPOB_EC_37 98 3821 F	TGGGCAGCGTTTCGGCG AAATGGA	245	RPOB_EC_386 2 3889 R	TGTCCGACTTGACGGTCAAG CATTCCTG	605
289	RPOB_EC_37 99 3821 F	GGGCAGCGTTTCGGCGA AATGGA	246	RPOB_EC_386 2 3888 R	GTCCGACTTGACGGTCAAC ATTTCCTG	602
362	RPOB_EC_37 99 3821 TM	TGGGCAGCGTTTCGGCG AAATGGA	245	RPOB_EC_386 2 3888 TMOD	TGTCCGACTTGACGGTCAA CATTCCTG	603



	OD_F			R		
288	RPOB_EC_38 02_3821_F	CAGCGTTTCGGCGAAAT GGA	247	RPOB_EC_386 2_3885_R	CGACTTGACGGTTAACATT TCCTG	601
48	RPOC_EC_10 18_1045_2_ F	CAAAACTTATTAGGTAA GCGTGTGACT	248	RPOC_EC_109 5_1124_2_R	TCAAGCGCCATCTCTTTTCG GTAATCCACAT	610
47	RPOC_EC_10 18_1045_F	CAAAACTTATTAGGTAA GCGTGTGACT	248	RPOC_EC_109 5_1124_R	TCAAGCGCCATTTCTTTTG GTAAACCACAT	611
68	RPOC_EC_10 36_1060_F	CGTGTGACTATTTCGGG GCGTTCAG	249	RPOC_EC_109 7_1126_R	ATTCAAGAGCCATTCTTTT TGGTAAACCAC	612
49	RPOC_EC_11 4_140_F	TAAGAAGCCGGAACCA TCAACTACCG	250	RPOC_EC_213 232_R	GGCGCTTGTACTTACCGCA C	617
227	RPOC_EC_12 56_1277_F	ACCCAGTGCTGCTGAAC CGTGC	251	RPOC_EC_129 5_1315_R	GTTCAAATGCCTGGATACC CA	613
292	RPOC_EC_13 74_1393_F	CGCCGACTTCGACGGTG ACC	252	RPOC_EC_143 7_1455_R	GAGCATCAGCGTGCGTGCT	614
364	RPOC_EC_13 74_1393_TM OD_F	TCGCCGACTTCGACGGT GACC	253	RPOC_EC_143 7_1455_TMOD R	TGAGCATCAGCGTGCGTGC T	615
229	RPOC_EC_15 84_1604_F	TGGCCCGAAAGAAGCTG AGCG	254	RPOC_EC_162 3_1643_R	ACGCGGGCATGCAGAGATG CC	616
978	RPOC_EC_21 45_2175_F	TCAGGAGTCGTTCAACT CGATCTACATGATG	255	RPOC_EC_222 8_2247_R	TTACGCCATCAGGCCACGC A	622
290	RPOC_EC_21 46_2174_F	CAGGAGTCGTTCAACTC GATCTACATGAT	256	RPOC_EC_222 7_2245_R	ACGCCATCAGGCCACGCAT	620
363	RPOC_EC_21 46_2174_TM OD_F	TCAGGAGTCGTTCAACT CGATCTACATGAT	257	RPOC_EC_222 7_2245_TMOD R	TACGCCATCAGGCCACGCA T	621
51	RPOC_EC_21 78_2196_2_ F	TGATTCCGGTGCCCCGTG GT	258	RPOC_EC_222 5_2246_2_R	TTGGCCATCAGACCACGCA TAC	618
50	RPOC_EC_21 78_2196_F	TGATTCTGGTGCCCCGTG GT	259	RPOC_EC_222 5_2246_R	TTGGCCATCAGGCCACGCA TAC	619
53	RPOC_EC_22 18_2241_2_ F	CTTGCTGGTATGCGTGG TCTGATG	260	RPOC_EC_231 3_2337_2_R	CGCACCATGCGTAGAGATG AAGTAC	623
52	RPOC_EC_22 18_2241_F	CTGGCAGGTATGCGTGG TCTGATG	261	RPOC_EC_231 3_2337_R	CGCACCCTGGGTTGAGATG AAGTAC	624
354	RPOC_EC_22 18_2241_TM OD_F	TCTGGCAGGTATGCGTG GTCTGATG	262	RPOC_EC_231 3_2337_TMOD R	TCGCACCCTGGGTTGAGAT GAAGTAC	625
958	RPOC_EC_22 23_2243_F	TGGTATGCGTGGTCTGA TGCC	263	RPOC_EC_232 9_2352_R	TGCTAGACCTTTACGTGCA CCGTG	626
960	RPOC_EC_23 34_2357_F	TGCTCGTAAGGGTCTGG CGGATAC	264	RPOC_EC_238 0_2403_R	TACTAGACGACGGGTCAGG TAACC	627
55	RPOC_EC_80 8_833_2_F	CGTCGTGTAATTAACCG TAACAACCG	265	RPOC_EC_865 891_R	ACGTTTTTCGTTTTGAACG ATAATGCT	629
54	RPOC_EC_80 8_833_F	CGTCGGGTGATTAACCG TAACAACCG	266	RPOC_EC_865 889_R	GTTTTTCGTTGCGTACGAT GATGTC	628
961	RPOC_EC_91 7_938_F	TATTGGACAACGGTCTGT CGCGG	267	RPOC_EC_100 9_1034_R	TTACCGAGCAGGTTCTGAC GGAAACG	607
959	RPOC_EC_91 8_938_F	TCTGGATAACGGTCGTC GCGG	268	RPOC_EC_100 9_1031_R	TCCAGCAGGTTCTGACGGA AACG	606
57	RPOC_EC_99 3_1019_2_F	CAAAGGTAAGCAAGGAC GTTCCGTCA	269	RPOC_EC_103 6_1059_2_R	CGAACGCCAGAGTAGTCA ACACG	608
56	RPOC_EC_99 3_1019_F	CAAAGGTAAGCAAGGTC GTTCCGTCA	270	RPOC_EC_103 6_1059_R	CGAACGCCCTGAGTAGTCA ACACG	609
75	SP101_SPET 11_1_29_F	AACCTTAATTGGAAAGA AACCCAAGAAGT	271	SP101_SPET1 1_92_116_R	CCTACCCAACGTTACACAA GGGCAG	676
446	SP101_SPET 11_1_29_TM OD_F	TAACCTTAATTGGAAAG AAACCCAAGAAGT	272	SP101_SPET1 1_92_116_TM OD_R	TCCTACCCAACGTTACCA AGGGCAG	677
85	SP101_SPET 11_1154_11 79_F	CAATACCGCAACAGCGG TGGCTTGGG	273	SP101_SPET1 1_1251_1277 R	GACCCCAACCTGGCCTTTT GTCGTGA	630
424	SP101_SPET 11_1154_11 79_TMOD_F	TCAATACCGCAACAGCG GTGGCTTGGG	274	SP101_SPET1 1_1251_1277 TMOD_R	TGACCCCAACCTGGCCTTT TGTCGTGA	631
76	SP101_SPET 11_118_147 F	GCTGGTGAAAATAACCC AGATGTCGTCTTC	275	SP101_SPET1 1_213_238_R	TGTGGCCGATTTACCAACC TGCTCCT	644
425	SP101_SPET 11_118_147 TMOD_F	TGCTGGTGAAAATAACC CAGATGTCGTCTTC	276	SP101_SPET1 1_213_238_T MOD_R	TTGTGGCCGATTTACCAAC CTGCTCCT	645
86	SP101_SPET	CGCAAAAAATCCAGCT	277	SP101_SPET1	AAACTATTTTTTTAGCTAT	632

	11_1314_13 36 F	ATTAGC		1_1403_1431 R	ACTCGAACAC	
426	SP101_SPET 11_1314_13 36 TMOD F	TCGCAAAAAAATCCAGC TATTAGC	278	SP101_SPET1 1_1403_1431 TMOD R	TAAACTATTTTTTTAGCTA TACTCGAACAC	633
87	SP101_SPET 11_1408_14 37 F	CGAGTATAGCTAAAAAA ATAGTTTATGACA	279	SP101_SPET1 1_1486_1515 R	GGATAATTGGTCGTAACAA GGGATAGTGAG	634
427	SP101_SPET 11_1408_14 37 TMOD F	TCGAGTATAGCTAAAAA AATAGTTTATGACA	280	SP101_SPET1 1_1486_1515 TMOD R	TGGATAATTGGTCGTAACA AGGGATAGTGAG	635
88	SP101_SPET 11_1688_17 16 F	CCTATATTAATCGTTTA CAGAACTGGCT	281	SP101_SPET1 1_1783_1808 R	ATATGATTATCATTGAACT GCGGCCG	636
428	SP101_SPET 11_1688_17 16 TMOD F	TCCTATATTAATCGTTT ACAGAACTGGCT	282	SP101_SPET1 1_1783_1808 TMOD R	TATATGATTATCATTGAAC TGCGGCCG	637
89	SP101_SPET 11_1711_17 33 F	CTGGCTAAACTTTGGC AACGGT	283	SP101_SPET1 1_1808_1835 R	GCGTGACGACCTTCTTGAA TTGTAATCA	638
429	SP101_SPET 11_1711_17 33 TMOD F	TCTGGCTAAACTTTGG CAACGGT	284	SP101_SPET1 1_1808_1835 TMOD R	TGCGTGACGACCTTCTTGA ATTGTAATCA	639
90	SP101_SPET 11_1807_18 35 F	ATGATTACAATTCAAGA AGGTCGTCACGC	285	SP101_SPET1 1_1901_1927 R	TTGGACCTGTAATCAGCTG AATACTGG	640
430	SP101_SPET 11_1807_18 35 TMOD F	TATGATTACAATTCAAG AAGGTCGTCACGC	286	SP101_SPET1 1_1901_1927 TMOD R	TTTGGACCTGTAATCAGCT GAATACTGG	641
91	SP101_SPET 11_1967_19 91 F	TAACGGTTATCATGGCC CAGATGGG	287	SP101_SPET1 1_2062_2083 R	ATTGCCCAGAAATCAAATC ATC	642
431	SP101_SPET 11_1967_19 91 TMOD F	TTAACGGTTATCATGGC CCAGATGGG	288	SP101_SPET1 1_2062_2083 TMOD R	TATTGCCCAGAAATCAAAT CATC	643
77	SP101_SPET 11_216_243 F	AGCAGGTGGTGAAATCG GCCACATGATT	289	SP101_SPET1 1_308_333 R	TGCCACTTTGACAACTCCT GTTGCTG	654
432	SP101_SPET 11_216_243 TMOD F	TAGCAGGTGGTGAAATC GGCCACATGATT	290	SP101_SPET1 1_308_333 T MOD R	TTGCCACTTTGACAACTCC TGTTGCTG	655
92	SP101_SPET 11_2260_22 83 F	CAGAGACCGTTTTATCC TATCAGC	291	SP101_SPET1 1_2375_2397 R	TCTGGGTGACCTGGTGTTC TAGA	646
433	SP101_SPET 11_2260_22 83 TMOD F	TCAGAGACCGTTTTATC CTATCAGC	292	SP101_SPET1 1_2375_2397 TMOD R	TTCTGGGTGACCTGGTGTTC TTAGA	647
93	SP101_SPET 11_2375_23 99 F	TCTAAACACCAGGTCA CCCAGAAG	293	SP101_SPET1 1_2470_2497 R	AGCTGCTAGATGAGCTTCT GCCATGGCC	648
434	SP101_SPET 11_2375_23 99 TMOD F	TTCTAAACACCAGGTC ACCCAGAAG	294	SP101_SPET1 1_2470_2497 TMOD R	TAGCTGCTAGATGAGCTTC TGCCATGGCC	649
94	SP101_SPET 11_2468_24 87 F	ATGGCCATGGCAGAAGC TCA	295	SP101_SPET1 1_2543_2570 R	CCATAAGGTCACCGTCACC ATTCAAAGC	650
435	SP101_SPET 11_2468_24 87 TMOD F	TATGGCCATGGCAGAAG CTCA	296	SP101_SPET1 1_2543_2570 TMOD R	TCCATAAGGTCACCGTCAC CATTCAAAGC	651
78	SP101_SPET 11_266_295 F	CTTGTTACTTGTGGCTCA CACGGCTGTTTGG	297	SP101_SPET1 1_355_380 R	GCTGCTTTGATGGCTGAAT CCCCCTTC	661
436	SP101_SPET 11_266_295 TMOD F	TCTTGTTACTTGTGGCTC ACACGGCTGTTTGG	298	SP101_SPET1 1_355_380 T MOD R	TGCTGCTTTGATGGCTGAA TCCCCCTTC	662
95	SP101_SPET 11_2961_29 84 F	ACCATGACAGAAGGCAT TTTGACA	299	SP101_SPET1 1_3023_3045 R	GGAATTTACCAGCGATAGA CACC	652
437	SP101_SPET 11_2961_29 84 TMOD F	TACCATGACAGAAGGCA TTTTGACA	300	SP101_SPET1 1_3023_3045 TMOD R	TGGAATTTACCAGCGATAG ACACC	653
96	SP101_SPET 11_3075_31 03 F	GATGACTTTTTAGCTAA TGGTCAGGCAGC	301	SP101_SPET1 1_3168_3196 R	AATCGACGACCATCTTGGA AAGATTCTC	656
438	SP101_SPET	TGATGACTTTTTAGCTA	302	SP101_SPET1	TAATCGACGACCATCTTGG	657

	11_3075_31 03 TMOD F	ATGGTCAGGCAGC		1_3168_3196 TMOD R	AAAGATTTCTC	
448	SP101_SPET 11_3085_31 04 F	TAGCTAATGGTCAGGCA GCC	303	SP101_SPET1 1_3170_3194 R	TCGACGACCATCTTGAAAA GATTTTC	658
79	SP101_SPET 11_322_344 F	GTCAAAAGTGGCACGTTT ACTGGC	304	SP101_SPET1 1_423_441 R	ATCCCCCTGCTTCTGCTGCC	665
439	SP101_SPET 11_322_344 TMOD F	TGTCAAAGTGGCACGTT TACTGGC	305	SP101_SPET1 1_423_441_T MOD R	TATCCCCCTGCTTCTGCTGC C	666
97	SP101_SPET 11_3386_34 03 F	AGCGTAAGGTGAACCT T	306	SP101_SPET1 1_3480_3506 R	CCAGCAGTTACTGTCCCCT CATCTTTG	659
440	SP101_SPET 11_3386_34 03 TMOD F	TAGCGTAAAGGTGAACCC TT	307	SP101_SPET1 1_3480_3506 TMOD R	TCCAGCAGTTACTGTCCCC TCATCTTTG	660
98	SP101_SPET 11_3511_35 35 F	GCTTCAGGAATCAATGA TGGAGCAG	308	SP101_SPET1 1_3605_3629 R	GGGTCTACACCTGCACCTTG CATAAC	663
441	SP101_SPET 11_3511_35 35 TMOD F	TGCTTCAGGAATCAATG ATGGAGCAG	309	SP101_SPET1 1_3605_3629 TMOD R	TGGGTCTACACCTGCACCTT GCATAAC	664
80	SP101_SPET 11_358_387 F	GGGGATTCAGCCATCAA AGCAGCTATTGAC	310	SP101_SPET1 1_448_473 R	CCAACCTTTTCCACAACAG AATCAGC	668
442	SP101_SPET 11_358_387 TMOD F	TGGGGATTAGCCATCA AAGCAGCTATTGAC	311	SP101_SPET1 1_448_473_T MOD R	TCCAACCTTTTCCACAACA GAATCAGC	669
447	SP101_SPET 11_364_385 F	TCAGCCATCAAAGCAGC TATTG	312	SP101_SPET1 1_448_471 R	TACCTTTTCCACAACAGAA TCAGC	667
81	SP101_SPET 11_600_629 F	CCTTACTTCGAATATG AATCTTTTGGAAG	313	SP101_SPET1 1_686_714 R	CCCATTTTTTTCACGCATGC TGAAAATATC	670
443	SP101_SPET 11_600_629 TMOD F	TCCTTACTTCGAATAT GAATCTTTTGGAAG	314	SP101_SPET1 1_686_714_T MOD R	TCCCATTTTTTTCACGCATG CTGAAAATATC	671
82	SP101_SPET 11_658_684 F	GGGGATTGATATCACCG ATAAGAAGAA	315	SP101_SPET1 1_756_784 R	GATTGGCGATAAAGTGATA TTTTCTAAAA	672
444	SP101_SPET 11_658_684 TMOD F	TGGGGATTGATATCACCC GATAAGAAGAA	316	SP101_SPET1 1_756_784_T MOD R	TGATTGGCGATAAAGTGAT ATTTTCTAAAA	673
83	SP101_SPET 11_776_801 F	TCGCCAATCAAACTAA GGGAATGGC	317	SP101_SPET1 1_871_896 R	GCCCACCAGAAAGACTAGC AGGATAA	674
445	SP101_SPET 11_776_801 TMOD F	TTCGCCAATCAAACTA AGGGAAATGGC	318	SP101_SPET1 1_871_896_T MOD R	TGCCACCAGAAAGACTAG CAGGATAA	675
84	SP101_SPET 11_893_921 F	GGGCAACAGCAGCGGAT TGCGATTGCGCG	319	SP101_SPET1 1_988_1012_ R	CATGACAGCCAAGACCTCA CCACC	678
423	SP101_SPET 11_893_921 TMOD F	TGGGCAACAGCAGCGGA TTGCGATTGCGCG	320	SP101_SPET1 1_988_1012_ TMOD R	TCATGACAGCCAAGACCTC ACCCACC	679
706	SSPE_BA_11 4_137 F	TCAAGCAAACGCACAAT CAGAAGC	321	SSPE_BA_196 222 R	TTGCACGTCTGTTTCAGTT GCAAATTC	683
612	SSPE_BA_11 4_137P F	TCAAGCAAACGCACAAC *U*AGAAGC	321	SSPE_BA_196 222P R	TTGCACGTU* <sup>c</sup> GTTTCAGT TGCAAATTC	684
58	SSPE_BA_11 5_137 F	CAAGCAAACGCACAATC AGAAGC	322	SSPE_BA_197 222 R	TGCACGTCTGTTTCAGTTG CAAATTC	686
355	SSPE_BA_11 5_137_TMOD F	TCAAGCAAACGCACAAT CAGAAGC	321	SSPE_BA_197 222 TMOD R	TTGCACGTCTGTTTCAGTT GCAAATTC	687
215	SSPE_BA_12 1_137 F	AACGCACAATCAGAAGC	323	SSPE_BA_197 216 R	TCTGTTTCAGTTGCAAATT C	685
699	SSPE_BA_12 3_153 F	TGCACAATCAGAAGCTA AGAAAGCGCAAGCT	324	SSPE_BA_202 231 R	TTTCACAGCATGCACGTCT GTTTCAGTTGC	688
704	SSPE_BA_14 6_168 F	TGCAAGCTTCTGGTGCT AGCATT	325	SSPE_BA_242 267 R	TTGTGATTGTTTTGCAGCT GATGTG	689
702	SSPE_BA_15 0_168 F	TGCTTCTGGTGCTAGCA TT	326	SSPE_BA_243 264 R	TGATTGTTTTGCAGCTGAT TGT	691
610	SSPE_BA_15 0_168P F	TGCTTCTGGC*GU* <sup>c</sup> AG U*ATT	326	SSPE_BA_243 264P R	TGATTGTTTTGU* <sup>c</sup> AGU* <sup>c</sup> TGA C* <sup>c</sup> GT	691

700	SSPE_BA_15 6 168 F	TGGTGTCTAGCATT	327	SSPE_BA_243 255 R	TGCAGCTGATTGT	690
608	SSPE_BA_15 6 168P F	TGGC <sup>a</sup> GU <sup>c</sup> AGU <sup>a</sup> ATT	327	SSPE_BA_243 255P R	TGU <sup>a</sup> AGU <sup>a</sup> TGAC <sup>c</sup> GT	690
705	SSPE_BA_63 89 F	TGCTAGTTATGGTACAG AGTTTTCGCAC	328	SSPE_BA_163 191 R	TCATAACTAGCATTGTGC TTTGAATGCT	682
703	SSPE_BA_72 89 F	TGGTACAGAGTTTGCGA C	329	SSPE_BA_163 182 R	TCATTGTGCTTTGAATGC T	681
611	SSPE_BA_72 89P F	TGGTAU <sup>a</sup> AGAGC <sup>a</sup> C <sup>c</sup> G U <sup>a</sup> GAC	329	SSPE_BA_163 182P R	TCATTGTGCC <sup>c</sup> C <sup>c</sup> GAAC <sup>a</sup> GU <sup>a</sup> T	681
701	SSPE_BA_75 89 F	TACAGAGTTTTCGCAC	330	SSPE_BA_163 177 R	TGTGCTTTGAATGCT	680
609	SSPE_BA_75 89P F	TAU <sup>a</sup> AGAGC <sup>a</sup> C <sup>c</sup> CGU <sup>a</sup> G AC	330	SSPE_BA_163 177P R	TGTGCC <sup>c</sup> C <sup>c</sup> GAAC <sup>a</sup> GU <sup>a</sup> T	680
1099	TOXR_VBC_1 35 158 F	TCGATTAGGCAGCAACG AAAGCCG	331	TOXR_VBC_22 1 246 R	TTCAAAACCTTGCTCTCGC CAAACAA	692
905	TRPE_AY094 355_1064_1 086 F	TCGACCTTTTGGCAGGAA CTAGAC	332	TRPE_AY0943 55_1171_119 6 R	TACATCGTTTCGCCCAAGA TCAATCA	693
904	TRPE_AY094 355_1278_1 303 F	TCAAATGTACAAGGTGA AGTGCGTGA	333	TRPE_AY0943 55_1392_141 8 R	TCCTCTTTTTCACAGGCTCT ACTTCATC	694
903	TRPE_AY094 355_1445_1 471 F	TGGATGGCATGGTGAAA TGGATATGTC	334	TRPE_AY0943 55_1551_158 0 R	TATTGGGGTTTCATTCCAC TCAGATTCTGG	695
902	TRPE_AY094 355_1467_1 491 F	ATGTCGATTGCAATCCG TACTTGTG	335	TRPE_AY0943 55_1569_159 2 R	TGCGCGAGCTTTTATTGG GTTTC	696
906	TRPE_AY094 355_666_68 8 F	GTGCATGCCGATACAGA GCAGAG	336	TRPE_AY0943 55_769_791_ R	TTCAAAATGCCGAGGCGTA TGTG	697
907	TRPE_AY094 355_757_77 6 F	TGCAAGCGCGACCACAT ACG	337	TRPE_AY0943 55_864_883_ R	TGCCCAGGTACAACCTGCA T	698
114	TUFB_EC_22 5 251 F	GCACTATGCACACGTAG ATTGTCTTG	338	TUFB_EC_284 309 R	TATAGCACCATCCATCTGA GCGGCAC	706
60	TUFB_EC_23 9 259 2 F	TTGACTGCCAGGTACAC GCTG	339	TUFB_EC_283 303 2 R	GCGTCCATTTGAGCAGCA CC	704
59	TUFB_EC_23 9 259 F	TAGACTGCCAGGACAC GCTG	340	TUFB_EC_283 303 R	GCGTCCATCTGAGCAGCA CC	705
942	TUFB_EC_25 1 278 F	TGCACGCCGACTATGTT AAGAATGAT	341	TUFB_EC_337 360 R	TATGTGCTCACGAGTTTGC GGCAT	707
941	TUFB_EC_27 5 299 F	TGATCACTGGTGCTGCT CAGATGGA	342	TUFB_EC_337 362 R	TGGATGTGCTCACGAGTCT GTGGCAT	708
117	TUFB_EC_75 7 774 F	AAGACGACCTGCACGGG C	343	TUFB_EC_849 867 R	GCGTCCACGTCTTCACGC	709
293	TUFB_EC_95 7 979 F	CCACACGCCGTCTTCA ACAAC	344	TUFB_EC_103 4 1058 R	GGCATCACCATTTCCTTGT CCTTCG	700
367	TUFB_EC_95 7 979_TMOD F	TCCACACGCCGTCTTTC AACAAC	345	TUFB_EC_103 4 1058_TMOD R	TGGCATCACCATTTCCTTGT TCCTTCG	701
62	TUFB_EC_97 6 1000 2 F	AACACCGTCTCAGTT CTACTTCC	346	TUFB_EC_104 5 1068 2 R	GTTGTCAACAGGCATTACC ATTTC	702
61	TUFB_EC_97 6 1000 F	AACACCGTCCGCGAGTT CTACTTCC	347	TUFB_EC_104 5 1068 R	GTTGTCCGAGGCATAACC ATTTC	703
63	TUFB_EC_98 5 1012 F	CCACAGTTCTACTCCG TACTACTGACG	348	TUFB_EC_103 3 1062 R	TCCAGGCATTACCATTCT ACTCCTTCTGG	699
225	VALS_EC_11 05 1124 F	CGTGGCGGCGTGGTTAT CGA	349	VALS_EC_119 5 1214 R	ACGAACGGATGTCGCGGT T	710
71	VALS_EC_11 05 1124 F	CGTGGCGGCGTGGTTAT CGA	349	VALS_EC_119 5 1218 R	CGGTACGAACGGATGTGCG CCGTT	711
358	VALS_EC_11 05 1124_TM OD F	TCGTGGCGGCGTGGTTA TCGA	350	VALS_EC_119 5 1218_TMOD R	TCGGTACGAACGGATGTGCG GCCGTT	712
965	VALS_EC_11 28 1151 F	TATGCTGACCGACCAGT GGTACGT	351	VALS_EC_123 1 1257 R	TTGCGCATCCAGGAGAAG TACATGTT	713
112	VALS_EC_18 33 1850 F	CGACGCGCTGCGCTTCA C	352	VALS_EC_192 0 1943 R	GCGTCCACAGCTTGTGTC AGAAG	714
116	VALS_EC_19 20 1943 F	CTTCTGCAACAAGCTGT GGAACGC	353	VALS_EC_194 8 1970 R	TCGCAGTTCATCAGCACGA AGCG	715
295	VALS_EC_61 0 649 F	ACCGAGCAAGGAGACCA GC	354	VALS_EC_705 727 R	TATAACGCACATCGTCAGG GTGA	716
931	WAAA_Z9692 5 2 29 F	TCTTGCTCTTTCGTGAG TTCAGTAAATG	355	WAAA_Z96925 115 138 R	CAAGCGGTTTGCTCAAAT AGTCA	717
932	WAAA_Z9692	TCGATCTGGTTTCATGC	356	WAAA_Z96925	TGGCACGAGCCTGACCTGT	718

	5_286_311_ F	TGTTTCAGT		_394_412_R		
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[0095] Primer pair name codes and reference sequences are shown in Table 2. The primer name code typically represents the gene to which the given primer pair is targeted. The primer pair name includes coordinates with respect to a reference sequence defined by an extraction of a section of sequence or defined by a GenBank gi number, or the corresponding complementary sequence of the extraction, or the entire GenBank gi number as indicated by the label “no extraction.” Where “no extraction” is indicated for a reference sequence, the coordinates of a primer pair named to the reference sequence are with respect to the GenBank gi listing. Gene abbreviations are shown in bold type in the “Gene Name” column.

**Table 2: Primer Name Codes and Reference Sequences**

Primer name code	Gene Name	Organism	Reference GenBank gi number	Extracted gene coordinates of gi number	Extraction or entire gene SEQ ID NO:
16S_EC	<b>16S</b> rRNA (16S ribosomal gene)	<i>Escherichia coli</i>	16127994	4033120..4034661	719
23S_EC	<b>23S</b> rRNA (23S ribosomal gene)	<i>Escherichia coli</i>	16127994	4166220..4169123	720
CAPC BA	<b>capC</b> (capsule biosynthesis gene)	<i>Bacillus anthracis</i>	6470151	Complement (55628..56074)	721
CYA BA	<b>cya</b> (cyclic AMP gene)	<i>Bacillus anthracis</i>	4894216	Complement (154288..156626)	722
DNAK_EC	<b>dnaK</b> (chaperone dnaK gene)	<i>Escherichia coli</i>	16127994	12163..14079	723
GROL_EC	<b>groL</b> (chaperonin groL)	<i>Escherichia coli</i>	16127994	4368603..4370249	724
HFLB_EC	<b>hflb</b> (cell division protein peptidase ftsH)	<i>Escherichia coli</i>	16127994	Complement (3322645..3324576)	725
INFB_EC	<b>infB</b> (protein chain initiation factor infB gene)	<i>Escherichia coli</i>	16127994	Complement (3310983..3313655)	726
LEF BA	<b>lef</b> (lethal factor)	<i>Bacillus anthracis</i>	21392688	Complement (149357..151786)	727
PAG BA	<b>pag</b> (protective antigen)	<i>Bacillus anthracis</i>	21392688	143779..146073	728
RPLB_EC	<b>rplB</b> (50S ribosomal protein L2)	<i>Escherichia coli</i>	16127994	3449001..3448180	729
RPOB_EC	<b>rpoB</b> (DNA-directed RNA polymerase beta chain)	<i>Escherichia coli</i>	6127994	Complement 4178823..4182851	730
RPOC_EC	<b>rpoC</b> (DNA-directed RNA polymerase beta' chain)	<i>Escherichia coli</i>	16127994	4182928..4187151	731
SP101ET_SPET_1_1	Concatenation comprising:  <b>gki</b> (glucose kinase)  <b>gtr</b> (glutamine transporter protein)  <b>murI</b> (glutamate racemase)  <b>mutS</b> (DNA mismatch	Artificial Sequence* - partial gene sequences of <i>Streptococcus pyogenes</i>	15674250	Complement (1258294..1258791)  complement (1236751..1237200)  312732..313169  Complement	732

	repair protein)  <b>xpt</b> (xanthine phosphoribosyl transferase)  <b>yqiL</b> (acetyl-CoA-acetyl transferase)  <b>tkt</b> (transketolase)			(1787602..1788007)  930977..931425  129471..129903  1391844..1391386	
SSPE BA	<b>sspE</b> (small acid-soluble spore protein)	<i>Bacillus anthracis</i>	30253828	226496..226783	733
TUFB EC	<b>tufB</b> (Elongation factor Tu)	<i>Escherichia coli</i>	16127994	4173523..4174707	734
VALS EC	<b>vals</b> (Valyl-tRNA synthetase)	<i>Escherichia coli</i>	16127994	Complement (4481405..4478550)	735
ASPS EC	<b>asps</b> (Aspartyl-tRNA synthetase)	<i>Escherichia coli</i>	16127994	complement (1946777..1948546)	736
CAF1 AF 053947	<b>caf1</b> (capsular protein caf1)	<i>Yersinia pestis</i>	2996286	No extraction - GenBank coordinates used	-
INV_U22 457	<b>inv</b> (invasin)	<i>Yersinia pestis</i>	1256565	74..3772	737
LL_NC00 3143	<i>Y. pestis</i> specific chromosomal genes - difference region	<i>Yersinia pestis</i>	16120353	No extraction - GenBank coordinates used	-
BONTA_X 52066	<b>BoNT/A</b> (neurotoxin type A)	<i>Clostridium botulinum</i>	40381	77..3967	738
MECA_Y1 4051	<b>mecA</b> methicillin resistance gene	<i>Staphylococcus aureus</i>	2791983	No extraction - GenBank coordinates used	739
TRPE AY 094355	<b>trpE</b> (anthranilate synthase (large component))	<i>Acinetobacter baumannii</i>	20853695	No extraction - GenBank coordinates used	740
RECA_AF 251469	<b>recA</b> (recombinase A)	<i>Acinetobacter baumannii</i>	9965210	No extraction - GenBank coordinates used	741
GYRA_AF 100557	<b>gyrA</b> (DNA gyrase subunit A)	<i>Acinetobacter baumannii</i>	4240540	No extraction - GenBank coordinates used	742
GYRB_AB 008700	<b>gyrB</b> (DNA gyrase subunit B)	<i>Acinetobacter baumannii</i>	4514436	No extraction - GenBank coordinates used	743
WAAA_Z9 6925	<b>waaA</b> (3-deoxy-D-manno-octulosonic-acid transferase)	<i>Acinetobacter baumannii</i>	2765828	No extraction - GenBank coordinates used	744
CJST_CJ	Concatenation comprising:  <b>tkt</b> (transketolase)  <b>glyA</b> (serine hydroxymethyltransferase)  <b>glta</b> (citrate synthase)  <b>aspA</b> (aspartate ammonia lyase)  <b>glnA</b> (glutamine synthase)  <b>pgm</b> (phosphoglycerate mutase)	Artificial Sequence* - partial gene sequences of <i>Campylobacter jejuni</i>	15791399	1569415..1569873  367573..368079  complement (1604529..1604930)  96692..97168  complement (657609..658085)  327773..328270	745

	<b>uncA</b> (ATP synthetase alpha chain)			112163..112651		
RNASEP_BDP	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Bordetella pertussis</i>	33591275	Complement (3226720..3227933)	746
RNASEP_BKM	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Burkholderia mallei</i>	53723370	Complement (2527296..2528220)	747
RNASEP_BS	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Bacillus subtilis</i>	16077068	Complement (2330250..2330962)	748
RNASEP_CLB	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Clostridium perfringens</i>	18308982	Complement (2291757..2292584)	749
RNASEP_EC	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Escherichia coli</i>	16127994	Complement (3267457..3268233)	750
RNASEP_RKP	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Rickettsia prowazekii</i>	15603881	complement(605276..606109)	751
RNASEP_SA	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Staphylococcus aureus</i>	15922990	complement(1559869..1560651)	752
RNASEP_VBC	<b>RNase</b> (ribonuclease P)	<b>P</b>	<i>Vibrio cholerae</i>	15640032	complement(2580367..2581452)	753
ICD_CXB	<b>icd</b> (isocitrate dehydrogenase)		<i>Coxiella burnetii</i>	29732244	complement(1143867..1144235)	754
	multi-locus IS1111A insertion element		<i>Acinetobacter baumannii</i>	29732244		-
IS1111A					No extraction	
OMPA_AY 485227	<b>ompA</b> (outer membrane protein A)		<i>Rickettsia prowazekii</i>	40287451	No extraction	755
OMP_B_RK P	<b>ompB</b> (outer membrane protein B)		<i>Rickettsia prowazekii</i>	15603881	complement(881264..886195)	756
GLTA_RK P	<b>glta</b> (citrate synthase)		<i>Vibrio cholerae</i>	15603881	complement(1062547..1063857)	757
TOXR_VB C	<b>toxR</b> (transcription regulator toxR)		<i>Francisella tularensis</i>	15640032	complement(1047143..1048024)	758
ASD_FRT	<b>asd</b> (Aspartate semialdehyde dehydrogenase)		<i>Francisella tularensis</i>	56707187	complement(438608..439702)	759
GALE_FR T	<b>galE</b> (UDP-glucose 4-epimerase)		<i>Shigella flexneri</i>	56707187	809039..810058	760
IPAH_SG F	<b>ipah</b> (invasion plasmid antigen)		<i>Campylobacter jejuni</i>	30061571	2210775..2211614	761
HUPB_CJ	<b>hupB</b> (DNA-binding protein H <sub>u</sub> -beta)		<i>Coxiella burnetii</i>	15791399	complement(849317..849819)	762
	Concatenation comprising :		Artificial Sequence* - partial gene sequences of <i>Acinetobacter baumannii</i>			763
AB_MLST	<b>trpE</b> (anthranilate synthase component I))  <b>adk</b> (adenylate kinase)  <b>mutY</b> (adenine glycosylase)  <b>fumC</b> (fumarate hydratase)  <b>efp</b> (elongation factor p)  <b>ppa</b> (pyrophosphate phospho-hydratase)		-		Sequenced in-house	

[0096] \* Note: These artificial reference sequences represent concatenations of partial gene extractions from the indicated reference gi number. Partial sequences were used to create the concatenated sequence because complete gene sequences were not necessary for primer design. The stretches of arbitrary residues "N"s were added for the convenience of separation of the partial gene extractions (100N for SP101\_SPET11 (SEQ ID NO: 732); 50N for CJST\_CJ (SEQ ID NO: 745); and 40N for AB\_MLST (SEQ ID NO: 763)).

**[0097] Example 2: DNA isolation and Amplification**

[0098] Genomic materials from culture samples or swabs were prepared using the DNeasy® 96 Tissue Kit (Qiagen, Valencia, CA). All PCR reactions are assembled in 50 µl reactions in the 96 well microtiter plate format using a Packard MPII liquid handling robotic platform and MJ Dyad® thermocyclers (MJ research, Waltham, MA). The PCR reaction consisted of 4 units of Amplitaq Gold®, 1x buffer II (Applied Biosystems, Foster City, CA), 1.5 mM MgCl<sub>2</sub>, 0.4 M betaine, 800 µM dNTP mix, and 250 nM of each primer.

[0099] The following PCR conditions were used to amplify the sequences used for mass spectrometry analysis: 95C for 10 minutes followed by 8 cycles of 95C for 30 seconds, 48C for 30 seconds, and 72C for 30 seconds, with the 48C annealing temperature increased 0.9C after each cycle. The PCR was then continued for 37 additional cycles of 95C for 15 seconds, 56C for 20 seconds, and 72C for 20 seconds.

**[0100] Example 3: Solution Capture Purification of PCR Products for Mass Spectrometry with Ion Exchange Resin-Magnetic Beads**

[0101] For solution capture of nucleic acids with ion exchange resin linked to magnetic beads, 25 µl of a 2.5 mg/mL suspension of BioClon amine terminated supraparamagnetic beads were added to 25 to 50 µl of a PCR reaction containing approximately 10 pM of a typical PCR amplification product. The above suspension was mixed for approximately 5 minutes by vortexing or pipetting, after which the liquid was removed after using a magnetic separator. The beads containing bound PCR amplification product were then washed 3x with 50mM ammonium bicarbonate/50% MeOH or 100mM ammonium bicarbonate/50% MeOH, followed by three more washes with 50% MeOH. The bound PCR amplicon was eluted with 25mM piperidine, 25mM imidazole, 35% MeOH, plus peptide calibration standards.



**[0102] Example 4: Mass Spectrometry and Base Composition Analysis**

**[0103]** The ESI-FTICR mass spectrometer is based on a Bruker Daltonics (Billerica, MA) Apex II 70e electrospray ionization Fourier transform ion cyclotron resonance mass spectrometer that employs an actively shielded 7 Tesla superconducting magnet. The active shielding constrains the majority of the fringing magnetic field from the superconducting magnet to a relatively small volume. Thus, components that might be adversely affected by stray magnetic fields, such as CRT monitors, robotic components, and other electronics, can operate in close proximity to the FTICR spectrometer. All aspects of pulse sequence control and data acquisition were performed on a 600 MHz Pentium II data station running Bruker's Xmass software under Windows NT 4.0 operating system. Sample aliquots, typically 1.5  $\mu$ l, were extracted directly from 96-well microtiter plates using a CTC HTS PAL autosampler (LEAP Technologies, Carrboro, NC) triggered by the FTICR data station. Samples were injected directly into a 10  $\mu$ l sample loop integrated with a fluidics handling system that supplies the 100  $\mu$ l/hr flow rate to the ESI source. Ions were formed via electrospray ionization in a modified Analytica (Branford, CT) source employing an off axis, grounded electrospray probe positioned approximately 1.5 cm from the metalized terminus of a glass desolvation capillary. The atmospheric pressure end of the glass capillary was biased at 6000 V relative to the ESI needle during data acquisition. A counter-current flow of dry N<sub>2</sub> was employed to assist in the desolvation process. Ions were accumulated in an external ion reservoir comprised of an rf-only hexapole, a skimmer cone, and an auxiliary gate electrode, prior to injection into the trapped ion cell where they were mass analyzed. Ionization duty cycles > 99% were achieved by simultaneously accumulating ions in the external ion reservoir during ion detection. Each detection event consisted of 1M data points digitized over 2.3 s. To improve the signal-to-noise ratio (S/N), 32 scans were co-added for a total data acquisition time of 74 s.

**[0104]** The ESI-TOF mass spectrometer is based on a Bruker Daltonics MicroTOF™. Ions from the ESI source undergo orthogonal ion extraction and are focused in a reflectron prior to detection. The TOF and FTICR are equipped with the same automated sample handling and fluidics described above. Ions are formed in the standard MicroTOF™ ESI source that is equipped with the same off-axis sprayer and glass capillary as the FTICR ESI source. Consequently, source conditions were the same as those described above. External ion accumulation was also employed to improve ionization duty cycle during data acquisition. Each detection event on the TOF was comprised of 75,000 data points digitized over 75  $\mu$ s.

[0105] The sample delivery scheme allows sample aliquots to be rapidly injected into the electrospray source at high flow rate and subsequently be electrosprayed at a much lower flow rate for improved ESI sensitivity. Prior to injecting a sample, a bolus of buffer was injected at a high flow rate to rinse the transfer line and spray needle to avoid sample contamination/carryover. Following the rinse step, the autosampler injected the next sample and the flow rate was switched to low flow. Following a brief equilibration delay, data acquisition commenced. As spectra were co-added, the autosampler continued rinsing the syringe and picking up buffer to rinse the injector and sample transfer line. In general, two syringe rinses and one injector rinse were required to minimize sample carryover. During a routine screening protocol a new sample mixture was injected every 106 seconds. More recently a fast wash station for the syringe needle has been implemented which, when combined with shorter acquisition times, facilitates the acquisition of mass spectra at a rate of just under one spectrum/minute.

[0106] Raw mass spectra were post-calibrated with an internal mass standard and deconvoluted to monoisotopic molecular masses. Unambiguous base compositions were derived from the exact mass measurements of the complementary single-stranded oligonucleotides. Quantitative results are obtained by comparing the peak heights with an internal PCR calibration standard present in every PCR well at 500 molecules per well for the ribosomal DNA-targeted primers and 100 molecules per well for the protein-encoding gene targets. Calibration methods are commonly owned and disclosed in U.S. Provisional Patent Application Serial No. 60/545,425.

**[0107] Example 5: *De Novo* Determination of Base Composition of Amplification Products using Molecular Mass Modified Deoxynucleotide Triphosphates**

[0108] Because the molecular masses of the four natural nucleobases have a relatively narrow molecular mass range (A = 313.058, G = 329.052, C = 289.046, T = 304.046 – See Table 3), a persistent source of ambiguity in assignment of base composition can occur as follows: two nucleic acid strands having different base composition may have a difference of about 1 Da when the base composition difference between the two strands is G ↔ A (-15.994) combined with C ↔ T (+15.000). For example, one 99-mer nucleic acid strand having a base composition of A<sub>27</sub>G<sub>30</sub>C<sub>21</sub>T<sub>21</sub> has a theoretical molecular mass of 30779.058 while another 99-mer nucleic acid strand having a base composition of A<sub>26</sub>G<sub>31</sub>C<sub>22</sub>T<sub>20</sub> has a theoretical molecular mass of 30780.052. A 1 Da difference in molecular mass may be within the experimental error of a

molecular mass measurement and thus, the relatively narrow molecular mass range of the four natural nucleobases imposes an uncertainty factor.

[0109] The present invention provides for a means for removing this theoretical 1 Da uncertainty factor through amplification of a nucleic acid with one mass-tagged nucleobase and three natural nucleobases. The term “nucleobase” as used herein is synonymous with other terms in use in the art including “nucleotide,” “deoxynucleotide,” “nucleotide residue,” “deoxynucleotide residue,” “nucleotide triphosphate (NTP),” or deoxynucleotide triphosphate (dNTP).

[0110] Addition of significant mass to one of the 4 nucleobases (dNTPs) in an amplification reaction, or in the primers themselves, will result in a significant difference in mass of the resulting amplification product (significantly greater than 1 Da) arising from ambiguities arising from the  $G \leftrightarrow A$  combined with  $C \leftrightarrow T$  event (Table 3). Thus, the same the  $G \leftrightarrow A$  (-15.994) event combined with 5-Iodo- $C \leftrightarrow T$  (-110.900) event would result in a molecular mass difference of 126.894. If the molecular mass of the base composition  $A_{27}G_{30}$  **5-Iodo- $C_{21}T_{21}$**  (33422.958) is compared with  $A_{26}G_{31}$  **5-Iodo- $C_{22}T_{20}$** , (33549.852) the theoretical molecular mass difference is +126.894. The experimental error of a molecular mass measurement is not significant with regard to this molecular mass difference. Furthermore, the only base composition consistent with a measured molecular mass of the 99-mer nucleic acid is  $A_{27}G_{30}$  **5-Iodo- $C_{21}T_{21}$** . In contrast, the analogous amplification without the mass tag has 18 possible base compositions.

**Table 3: Molecular Masses of Natural Nucleobases and the Mass-Modified Nucleobase 5-Iodo-C and Molecular Mass Differences Resulting from Transitions**

Nucleobase	Molecular Mass	Transition	$\Delta$ Molecular Mass
A	313.058	A $\rightarrow$ T	-9.012
A	313.058	A $\rightarrow$ C	-24.012
A	313.058	A $\rightarrow$ 5-Iodo-C	101.888
A	313.058	A $\rightarrow$ G	15.994
T	304.046	T $\rightarrow$ A	9.012
T	304.046	T $\rightarrow$ C	-15.000
T	304.046	T $\rightarrow$ 5-Iodo-C	110.900
T	304.046	T $\rightarrow$ G	25.006
C	289.046	C $\rightarrow$ A	24.012
C	289.046	C $\rightarrow$ T	15.000
C	289.046	C $\rightarrow$ G	40.006

5-Iodo-C	414.946	5-Iodo-C-->A	-101.888
5-Iodo-C	414.946	5-Iodo-C-->T	-110.900
5-Iodo-C	414.946	5-Iodo-C-->G	-85.894
G	329.052	G-->A	-15.994
G	329.052	G-->T	-25.006
G	329.052	G-->C	-40.006
G	329.052	G-->5-Iodo-C	85.894

### [0111] Example 6: Data Processing

[0112] Mass spectra of bioagent identifying amplicons are analyzed independently using a maximum-likelihood processor, such as is widely used in radar signal processing. This processor, referred to as GenX, first makes maximum likelihood estimates of the input to the mass spectrometer for each primer by running matched filters for each base composition aggregate on the input data. This includes the GenX response to a calibrant for each primer.

[0113] The algorithm emphasizes performance predictions culminating in probability-of-detection versus probability-of-false-alarm plots for conditions involving complex backgrounds of naturally occurring organisms and environmental contaminants. Matched filters consist of *a priori* expectations of signal values given the set of primers used for each of the bioagents. A genomic sequence database is used to define the mass base count matched filters. The database contains the sequences of known bacterial bioagents and includes threat organisms as well as benign background organisms. The latter is used to estimate and subtract the spectral signature produced by the background organisms. A maximum likelihood detection of known background organisms is implemented using matched filters and a running-sum estimate of the noise covariance. Background signal strengths are estimated and used along with the matched filters to form signatures which are then subtracted. the maximum likelihood process is applied to this "cleaned up" data in a similar manner employing matched filters for the organisms and a running-sum estimate of the noise-covariance for the cleaned up data.

[0114] The amplitudes of all base compositions of bioagent identifying amplicons for each primer are calibrated and a final maximum likelihood amplitude estimate per organism is made based upon the multiple single primer estimates. Models of all system noise are factored into this two-stage maximum likelihood calculation. The processor reports the number of molecules of each base composition contained in the spectra. The quantity of amplification product

corresponding to the appropriate primer set is reported as well as the quantities of primers remaining upon completion of the amplification reaction.

**[0115] Example 7: Use of Broad Range Survey and Division Wide Primer Pairs for Identification of Bacteria in an Epidemic Surveillance Investigation**

[0116] This investigation employed a set of 16 primer pairs which is herein designated the “surveillance primer set” and comprises broad range survey primer pairs, division wide primer pairs and a single *Bacillus* clade primer pair. The surveillance primer set is shown in Table 4 and consists of primer pairs originally listed in Table 1. This surveillance set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row. Primer pair 449 (non-T modified) has been modified twice. Its predecessors are primer pairs 70 and 357, displayed below in the same row. Primer pair 360 has also been modified twice and its predecessors are primer pairs 17 and 118.

**Table 4: Bacterial Primer Pairs of the Surveillance Primer Set**

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
346	16S_EC_713_732_TMOD_F	27	16S_EC_789_809_TMOD_R	389	16S rRNA
10	16S_EC_713_732_F	26	16S_EC_789_809	388	16S rRNA
347	16S_EC_785_806_TMOD_F	30	16S_EC_880_897_TMOD_R	392	16S rRNA
11	16S_EC_785_806_F	29	16S_EC_880_897_R	391	16S rRNA
348	16S_EC_960_981_TMOD_F	38	16S_EC_1054_1073_TMOD_R	363	16S rRNA
14	16S_EC_960_981_F	37	16S_EC_1054_1073_R	362	16S rRNA
349	23S_EC_1826_1843_TMOD_F	49	23S_EC_1906_1924_TMOD_R	405	23S rRNA
16	23S_EC_1826_1843_F	48	23S_EC_1906_1924_R	404	23S rRNA
352	INFB_EC_1365_1393_TMOD_F	161	INFB_EC_1439_1467_TMOD_R	516	infB
34	INFB_EC_1365_1393_F	160	INFB_EC_1439_1467_R	515	infB
354	RPOC_EC_2218_2241_TMOD_F	262	RPOC_EC_2313_2337_TMOD_R	625	rpoC
52	RPOC_EC_2218_2241_F	261	RPOC_EC_2313_2337_R	624	rpoC
355	SSPE_BA_115_137_TMOD_F	321	SSPE_BA_197_222_TMOD_R	687	sspE
58	SSPE_BA_115_137_F	322	SSPE_BA_197_222_R	686	sspE
356	RPLB_EC_650_679_TMOD_F	232	RPLB_EC_739_762_TMOD_R	592	rplB
66	RPLB_EC_650_679_F	231	RPLB_EC_739_762_R	591	rplB
358	VALS_EC_1105_1124_TMOD_F	350	VALS_EC_1195_1218_TMOD_R	712	valS
71	VALS_EC_1105_1124_F	349	VALS_EC_1195_1218_R	711	valS
359	RPOB_EC_1845_1866_TMOD_F	241	RPOB_EC_1909_1929_TMOD_R	597	rpoB
72	RPOB_EC_1845_1866_F	240	RPOB_EC_1909_1929_R	596	rpoB
360	23S_EC_2646_2667_TMOD_F	60	23S_EC_2745_2765_TMOD_R	416	23S rRNA
118	23S_EC_2646_2667_F	59	23S_EC_2745_2765_R	415	23S rRNA
17	23S_EC_2645_2669_F	58	23S_EC_2744_2761_R	414	23S rRNA

361	16S_EC_1090_1111_2_TM0D_F	5	16S_EC_1175_1196_TM0D_R	370	16S rRNA
3	16S_EC_1090_1111_2_F	6	16S_EC_1175_1196_R	369	16S rRNA
362	RPOB_EC_3799_3821_TM0D_F	245	RPOB_EC_3862_3888_TM0D_R	603	rpoB
289	RPOB_EC_3799_3821_F	246	RPOB_EC_3862_3888_R	602	rpoB
363	RPOC_EC_2146_2174_TM0D_F	257	RPOC_EC_2227_2245_TM0D_R	621	rpoC
290	RPOC_EC_2146_2174_F	256	RPOC_EC_2227_2245_R	620	rpoC
367	TUFB_EC_957_979_TM0D_F	345	TUFB_EC_1034_1058_TM0D_R	701	tufB
293	TUFB_EC_957_979_F	344	TUFB_EC_1034_1058_R	700	tufB
449	RPLB_EC_690_710_F	237	RPLB_EC_737_758_R	589	rplB
357	RPLB_EC_688_710_TM0D_F	236	RPLB_EC_736_757_TM0D_R	588	rplB
67	RPLB_EC_688_710_F	235	RPLB_EC_736_757_R	587	rplB

[0117] The 16 primer pairs of the surveillance set are used to produce bioagent identifying amplicons whose base compositions are sufficiently different amongst all known bacteria at the species level to identify, at a reasonable confidence level, any given bacterium at the species level. As shown in Tables 6A-E, common respiratory bacterial pathogens can be distinguished by the base compositions of bioagent identifying amplicons obtained using the 16 primer pairs of the surveillance set. In some cases, triangulation identification improves the confidence level for species assignment. For example, nucleic acid from *Streptococcus pyogenes* can be amplified by nine of the sixteen surveillance primer pairs and *Streptococcus pneumoniae* can be amplified by ten of the sixteen surveillance primer pairs. The base compositions of the bioagent identifying amplicons are identical for only one of the analogous bioagent identifying amplicons and differ in all of the remaining analogous bioagent identifying amplicons by up to four bases per bioagent identifying amplicon. The resolving power of the surveillance set was confirmed by determination of base compositions for 120 isolates of respiratory pathogens representing 70 different bacterial species and the results indicated that natural variations (usually only one or two base substitutions per bioagent identifying amplicon) amongst multiple isolates of the same species did not prevent correct identification of major pathogenic organisms at the species level.

[0118] *Bacillus anthracis* is a well known biological warfare agent which has emerged in domestic terrorism in recent years. Since it was envisioned to produce bioagent identifying amplicons for identification of *Bacillus anthracis*, additional drill-down analysis primers were designed to target genes present on virulence plasmids of *Bacillus anthracis* so that additional confidence could be reached in positive identification of this pathogenic organism. Three drill-down analysis primers were designed and are listed in Tables 1 and 5. In Table 5 the drill-down set comprises primers with T modifications (note TMOD designation in primer names) which

constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row.

**Table 5: Drill-Down Primer Pairs for Confirmation of Identification of *Bacillus anthracis***

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
350	CAPC_BA_274_303_TMOD_F	98	CAPC_BA_349_376_TMOD_R	452	capC
24	CAPC_BA_274_303_F	97	CAPC_BA_349_376_R	451	capC
351	CYA_BA_1353_1379_TMOD_F	128	CYA_BA_1448_1467_TMOD_R	483	cyA
30	CYA_BA_1353_1379_F	127	CYA_BA_1448_1467_R	482	cyA
353	LEF_BA_756_781_TMOD_F	175	LEF_BA_843_872_TMOD_R	531	lef
37	LEF_BA_756_781_F	174	LEF_BA_843_872_R	530	lef

[0119] Phylogenetic coverage of bacterial space of the sixteen surveillance primers of Table 4 and the three *Bacillus anthracis* drill-down primers of Table 5 is shown in Figure 3 which lists common pathogenic bacteria. Figure 3 is not meant to be comprehensive in illustrating all species identified by the primers. Only pathogenic bacteria are listed as representative examples of the bacterial species that can be identified by the primers and methods of the present invention. Nucleic acid of groups of bacteria enclosed within the polygons of Figure 3 can be amplified to obtain bioagent identifying amplicons using the primer pair numbers listed in the upper right hand corner of each polygon. Primer coverage for polygons within polygons is additive. As an illustrative example, bioagent identifying amplicons can be obtained for *Chlamydia trachomatis* by amplification with, for example, primer pairs 346-349, 360 and 361, but not with any of the remaining primers of the surveillance primer set. On the other hand, bioagent identifying amplicons can be obtained from nucleic acid originating from *Bacillus anthracis* (located within 5 successive polygons) using, for example, any of the following primer pairs: 346-349, 360, 361 (base polygon), 356, 449 (second polygon), 352 (third polygon), 355 (fourth polygon), 350, 351 and 353 (fifth polygon). Multiple coverage of a given organism with multiple primers provides for increased confidence level in identification of the organism as a result of enabling broad triangulation identification.

[0120] In Tables 6A-E, base compositions of respiratory pathogens for primer target regions are shown. Two entries in a cell, represent variation in ribosomal DNA operons. The most predominant base composition is shown first and the minor (frequently a single operon) is indicated by an asterisk (\*). Entries with NO DATA mean that the primer would not be expected to prime this species due to mismatches between the primer and target region, as determined by theoretical PCR.

**Table 6A – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 346, 347 and 348**

Organism	Strain	Primer 346 [A G C T]	Primer 347 [A G C T]	Primer 348 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	[29 32 25 13] [29 31 25 13]*	[23 38 28 26] [23 37 28 26]*	[26 32 28 30] [26 31 28 30]*
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	[29 32 25 13]	[22 39 28 26]	[29 30 28 29] [30 30 27 29]*
<i>Yersinia pestis</i>	KIM5 P12 (Biovar Mediaevalis)	[29 32 25 13]	[22 39 28 26]	[29 30 28 29]
<i>Yersinia pestis</i>	91001	[29 32 25 13]	[22 39 28 26]	[29 30 28 29] [30 30 27 29]*
<i>Haemophilus influenzae</i>	KW20	[28 31 23 17]	[24 37 25 27]	[29 30 28 29]
<i>Pseudomonas aeruginosa</i>	PAO1	[30 31 23 15]	[26 36 29 24] [27 36 29 23]*	[26 32 29 29]
<i>Pseudomonas fluorescens</i>	Pf0-1	[30 31 23 15]	[26 35 29 25]	[28 31 28 29]
<i>Pseudomonas putida</i>	KT2440	[30 31 23 15]	[28 33 27 27]	[27 32 29 28]
<i>Legionella pneumophila</i>	Philadelphia-1	[30 30 24 15]	[33 33 23 27]	[29 28 28 31]
<i>Francisella tularensis</i>	schu 4	[32 29 22 16]	[28 38 26 26]	[25 32 28 31]
<i>Bordetella pertussis</i>	Tohama I	[30 29 24 16]	[23 37 30 24]	[30 32 30 26]
<i>Burkholderia cepacia</i>	J2315	[29 29 27 14]	[27 32 26 29]	[27 36 31 24] [20 42 35 19]*
<i>Burkholderia pseudomallei</i>	K96243	[29 29 27 14]	[27 32 26 29]	[27 36 31 24]
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	[29 28 24 18]	[27 34 26 28]	[24 36 29 27]
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
<i>Neisseria meningitidis</i>	serogroup C, FAM18	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	[29 28 26 16]	[27 34 27 27]	[25 35 30 26]
<i>Chlamydophila pneumoniae</i>	TW-183	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Chlamydophila pneumoniae</i>	AR39	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Chlamydophila pneumoniae</i>	CWL029	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Chlamydophila pneumoniae</i>	J138	[31 27 22 19]	NO DATA	[32 27 27 29]
<i>Corynebacterium diphtheriae</i>	NCTC13129	[29 34 21 15]	[22 38 31 25]	[22 33 25 34]
<i>Mycobacterium avium</i>	k10	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium avium</i>	104	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium tuberculosis</i>	CSU#93	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium tuberculosis</i>	CDC 1551	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	[27 36 21 15]	[22 37 30 28]	[21 36 27 30]
<i>Mycoplasma pneumoniae</i>	M129	[31 29 19 20]	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [29 31 30 29]*
<i>Staphylococcus aureus</i>	MSSA476	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
<i>Staphylococcus aureus</i>	COL	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
<i>Staphylococcus aureus</i>	Mu50	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
<i>Staphylococcus aureus</i>	MW2	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*



<i>Staphylococcus aureus</i>	N315	[27 30 21 21]	[25 35 30 26]	[30 29 30 29] [30 29 29 30]*
<i>Staphylococcus aureus</i>	NCTC 8325	[27 30 21 21]	[25 35 30 26] [25 35 31 26]*	[30 29 30 29] [30 29 29 30]
<i>Streptococcus agalactiae</i>	NEM316	[26 32 23 18]	[24 36 31 25] [24 36 30 26]*	[25 32 29 30]
<i>Streptococcus equi</i>	NC 002955	[26 32 23 18]	[23 37 31 25]	[29 30 25 32]
<i>Streptococcus pyogenes</i>	MGAS8232	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	MGAS315	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	SSI-1	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	MGAS10394	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	Manfredo (M5)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pyogenes</i>	SF370 (M1)	[26 32 23 18]	[24 37 30 25]	[25 31 29 31]
<i>Streptococcus pneumoniae</i>	670	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
<i>Streptococcus pneumoniae</i>	R6	[26 32 23 18]	[25 35 28 28]	[25 32 29 30]
<i>Streptococcus pneumoniae</i>	TIGR4	[26 32 23 18]	[25 35 28 28]	[25 32 30 29]
<i>Streptococcus gordonii</i>	NCTC7868	[25 33 23 18]	[24 36 31 25]	[25 31 29 31]
<i>Streptococcus mitis</i>	NCTC 12261	[26 32 23 18]	[25 35 30 26]	[25 32 29 30] [24 31 35 29]*
<i>Streptococcus mutans</i>	UA159	[24 32 24 19]	[25 37 30 24]	[28 31 26 31]

**Table 6B – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 349, 360, and 356**

Organism	Strain	Primer 349 [A G C T]	Primer 360 [A G C T]	Primer 356 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	[25 31 25 22]	[33 37 25 27]	NO DATA
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	[25 31 27 20] [25 32 26 20]*	[34 35 25 28]	NO DATA
<i>Yersinia pestis</i>	KIM5 P12 (Biovar Mediaevalis)	[25 31 27 20] [25 32 26 20]*	[34 35 25 28]	NO DATA
<i>Yersinia pestis</i>	91001	[25 31 27 20]	[34 35 25 28]	NO DATA
<i>Haemophilus influenzae</i>	KW20	[28 28 25 20]	[32 38 25 27]	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	[24 31 26 20]	[31 36 27 27] [31 36 27 28]*	NO DATA
<i>Pseudomonas fluorescens</i>	Pf0-1	NO DATA	[30 37 27 28] [30 37 27 28]	NO DATA
<i>Pseudomonas putida</i>	KT2440	[24 31 26 20]	[30 37 27 28]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	[23 30 25 23]	[30 39 29 24]	NO DATA
<i>Francisella tularensis</i>	schu 4	[26 31 25 19]	[32 36 27 27]	NO DATA
<i>Bordetella pertussis</i>	Tohama I	[21 29 24 18]	[33 36 26 27]	NO DATA
<i>Burkholderia cepacia</i>	J2315	[23 27 22 20]	[31 37 28 26]	NO DATA
<i>Burkholderia pseudomallei</i>	K96243	[23 27 22 20]	[31 37 28 26]	NO DATA
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	[24 27 24 17]	[34 37 25 26]	NO DATA
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	[25 27 22 18]	[34 37 25 26]	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	[25 26 23 18]	[34 37 25 26]	NO DATA
<i>Neisseria</i>	Z2491 (serogroup A)	[25 26 23 18]	[34 37 25 26]	NO DATA

<i>meningitidis</i>				
<i>Chlamydomydia pneumoniae</i>	TW-183	[30 28 27 18]	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	AR39	[30 28 27 18]	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	CWL029	[30 28 27 18]	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	J138	[30 28 27 18]	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	[29 40 28 25]	NO DATA
<i>Mycobacterium avium</i>	k10	NO DATA	[33 35 32 22]	NO DATA
<i>Mycobacterium avium</i>	104	NO DATA	[33 35 32 22]	NO DATA
<i>Mycobacterium tuberculosis</i>	CSU#93	NO DATA	[30 36 34 22]	NO DATA
<i>Mycobacterium tuberculosis</i>	CDC 1551	NO DATA	[30 36 34 22]	NO DATA
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	NO DATA	[30 36 34 22]	NO DATA
<i>Mycoplasma pneumoniae</i>	M129	[28 30 24 19]	[34 31 29 26]	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	MSSA476	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	COL	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	Mu50	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	MW2	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	N315	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Staphylococcus aureus</i>	NCTC 8325	[26 30 25 20]	[31 38 24 29]	[33 30 31 27]
<i>Streptococcus agalactiae</i>	NEM316	[28 31 22 20]	[33 37 24 28]	[37 30 28 26]
<i>Streptococcus equi</i>	NC 002955	[28 31 23 19]	[33 38 24 27]	[37 31 28 25]
<i>Streptococcus pyogenes</i>	MGAS8232	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	MGAS315	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	SSI-1	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	MGAS10394	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	Manfredo (M5)	[28 31 23 19]	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pyogenes</i>	SF370 (M1)	[28 31 23 19] [28 31 22 20]*	[33 37 24 28]	[38 31 29 23]
<i>Streptococcus pneumoniae</i>	670	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus pneumoniae</i>	R6	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus pneumoniae</i>	TIGR4	[28 31 22 20]	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus gordonii</i>	NCTC7868	[28 32 23 20]	[34 36 24 28]	[36 31 29 25]
<i>Streptococcus mitis</i>	NCTC 12261	[28 31 22 20] [29 30 22 20]*	[34 36 24 28]	[37 30 29 25]
<i>Streptococcus mutans</i>	UA159	[26 32 23 22]	[34 37 24 27]	NO DATA

**Table 6C – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 449, 354, and 352**

Organism	Strain	Primer 449 [A G C T]	Primer 354 [A G C T]	Primer 352 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	NO DATA	[27 33 36 26]	NO DATA
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	NO DATA	[29 31 33 29]	[32 28 20 25]
<i>Yersinia pestis</i>	KIM5 P12 (Biovar Mediaevalis)	NO DATA	[29 31 33 29]	[32 28 20 25]
<i>Yersinia pestis</i>	91001	NO DATA	[29 31 33 29]	NO DATA
<i>Haemophilus influenzae</i>	KW20	NO DATA	[30 29 31 32]	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	NO DATA	[26 33 39 24]	NO DATA
<i>Pseudomonas fluorescens</i>	Pf0-1	NO DATA	[26 33 34 29]	NO DATA
<i>Pseudomonas putida</i>	KT2440	NO DATA	[25 34 36 27]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	NO DATA	NO DATA	NO DATA
<i>Francisella tularensis</i>	schu 4	NO DATA	[33 32 25 32]	NO DATA
<i>Bordetella pertussis</i>	Tohama I	NO DATA	[26 33 39 24]	NO DATA
<i>Burkholderia cepacia</i>	J2315	NO DATA	[25 37 33 27]	NO DATA
<i>Burkholderia pseudomallei</i>	K96243	NO DATA	[25 37 34 26]	NO DATA
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	[17 23 22 10]	[29 31 32 30]	NO DATA
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	NO DATA	[29 30 32 31]	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	NO DATA	[29 30 32 31]	NO DATA
<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	NO DATA	[29 30 32 31]	NO DATA
<i>Chlamydomphila pneumoniae</i>	TW-183	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	AR39	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	CWL029	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	J138	NO DATA	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	k10	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	104	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CSU#93	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CDC 1551	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
<i>Mycoplasma pneumoniae</i>	M129	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	MSSA476	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	COL	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
<i>Staphylococcus aureus</i>	Mu50	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	MW2	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]

<i>Staphylococcus aureus</i>	N315	[17 20 21 17]	[30 27 30 35]	[36 24 19 26]
<i>Staphylococcus aureus</i>	NCTC 8325	[17 20 21 17]	[30 27 30 35]	[35 24 19 27]
<i>Streptococcus agalactiae</i>	NEM316	[22 20 19 14]	[26 31 27 38]	[29 26 22 28]
<i>Streptococcus equi</i>	NC 002955	[22 21 19 13]	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS8232	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	MGAS315	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	SSI-1	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	MGAS10394	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	Manfredo (M5)	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pyogenes</i>	SF370 (M1)	[23 21 19 12]	[24 32 30 36]	NO DATA
<i>Streptococcus pneumoniae</i>	670	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
<i>Streptococcus pneumoniae</i>	R6	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
<i>Streptococcus pneumoniae</i>	TIGR4	[22 20 19 14]	[25 33 29 35]	[30 29 21 25]
<i>Streptococcus gordonii</i>	NCTC7868	[21 21 19 14]	NO DATA	[29 26 22 28]
<i>Streptococcus mitis</i>	NCTC 12261	[22 20 19 14]	[26 30 32 34]	NO DATA
<i>Streptococcus mutans</i>	UA159	NO DATA	NO DATA	NO DATA

**Table 6D – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 355, 358, and 359**

Organism	Strain	Primer 355 [A G C T]	Primer 358 [A G C T]	Primer 359 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	NO DATA	[24 39 33 20]	[25 21 24 17]
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	NO DATA	[26 34 35 21]	[23 23 19 22]
<i>Yersinia pestis</i>	KIM5 P12 (Biovar Mediaevalis)	NO DATA	[26 34 35 21]	[23 23 19 22]
<i>Yersinia pestis</i>	91001	NO DATA	[26 34 35 21]	[23 23 19 22]
<i>Haemophilus influenzae</i>	KW20	NO DATA	NO DATA	NO DATA
<i>Pseudomonas aeruginosa</i>	PA01	NO DATA	NO DATA	NO DATA
<i>Pseudomonas fluorescens</i>	Pf0-1	NO DATA	NO DATA	NO DATA
<i>Pseudomonas putida</i>	KT2440	NO DATA	[21 37 37 21]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	NO DATA	NO DATA	NO DATA
<i>Francisella tularensis</i>	schu 4	NO DATA	NO DATA	NO DATA
<i>Bordetella pertussis</i>	Tohama I	NO DATA	NO DATA	NO DATA
<i>Burkholderia cepacia</i>	J2315	NO DATA	NO DATA	NO DATA
<i>Burkholderia pseudomallei</i>	K96243	NO DATA	NO DATA	NO DATA
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	NO DATA	NO DATA	NO DATA

<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	TW-183	NO DATA	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	AR39	NO DATA	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	CWL029	NO DATA	NO DATA	NO DATA
<i>Chlamydomydia pneumoniae</i>	J138	NO DATA	NO DATA	NO DATA
<i>Corynebacterium diptheriae</i>	NCTC13129	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	k10	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	104	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CSU#93	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	CDC 1551	NO DATA	NO DATA	NO DATA
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	NO DATA	NO DATA	NO DATA
<i>Mycoplasma pneumoniae</i>	M129	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MSSA476	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	COL	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	Mu50	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MW2	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	N315	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	NCTC 8325	NO DATA	NO DATA	NO DATA
<i>Streptococcus agalactiae</i>	NEM316	NO DATA	NO DATA	NO DATA
<i>Streptococcus equi</i>	NC 002955	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS8232	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS315	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SSI-1	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS10394	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	Manfredo (M5)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SF370 (M1)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	670	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	R6	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	TIGR4	NO DATA	NO DATA	NO DATA
<i>Streptococcus gordonii</i>	NCTC7868	NO DATA	NO DATA	NO DATA
<i>Streptococcus mitis</i>	NCTC 12261	NO DATA	NO DATA	NO DATA
<i>Streptococcus mutans</i>	UA159	NO DATA	NO DATA	NO DATA

**Table 6E – Base Compositions of Common Respiratory Pathogens for Bioagent Identifying Amplicons Corresponding to Primer Pair Nos: 362, 363, and 367**

Organism	Strain	Primer 362 [A G C T]	Primer 363 [A G C T]	Primer 367 [A G C T]
<i>Klebsiella pneumoniae</i>	MGH78578	[21 33 22 16]	[16 34 26 26]	NO DATA
<i>Yersinia pestis</i>	CO-92 Biovar Orientalis	[20 34 18 20]	NO DATA	NO DATA
<i>Yersinia pestis</i>	KIM5 P12 (Biovar Mediaevalis)	[20 34 18 20]	NO DATA	NO DATA
<i>Yersinia pestis</i>	91001	[20 34 18 20]	NO DATA	NO DATA
<i>Haemophilus influenzae</i>	KW20	NO DATA	NO DATA	NO DATA
<i>Pseudomonas aeruginosa</i>	PAO1	[19 35 21 17]	[16 36 28 22]	NO DATA
<i>Pseudomonas fluorescens</i>	Pf0-1	NO DATA	[18 35 26 23]	NO DATA
<i>Pseudomonas putida</i>	KT2440	NO DATA	[16 35 28 23]	NO DATA
<i>Legionella pneumophila</i>	Philadelphia-1	NO DATA	NO DATA	NO DATA
<i>Francisella tularensis</i>	schu 4	NO DATA	NO DATA	NO DATA
<i>Bordetella pertussis</i>	Tohama I	[20 31 24 17]	[15 34 32 21]	[26 25 34 19]
<i>Burkholderia cepacia</i>	J2315	[20 33 21 18]	[15 36 26 25]	[25 27 32 20]
<i>Burkholderia pseudomallei</i>	K96243	[19 34 19 20]	[15 37 28 22]	[25 27 32 20]
<i>Neisseria gonorrhoeae</i>	FA 1090, ATCC 700825	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	MC58 (serogroup B)	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	serogroup C, FAM18	NO DATA	NO DATA	NO DATA
<i>Neisseria meningitidis</i>	Z2491 (serogroup A)	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	TW-183	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	AR39	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	CWL029	NO DATA	NO DATA	NO DATA
<i>Chlamydomphila pneumoniae</i>	J138	NO DATA	NO DATA	NO DATA
<i>Corynebacterium diphtheriae</i>	NCTC13129	NO DATA	NO DATA	NO DATA
<i>Mycobacterium avium</i>	k10	[19 34 23 16]	NO DATA	[24 26 35 19]
<i>Mycobacterium avium</i>	104	[19 34 23 16]	NO DATA	[24 26 35 19]
<i>Mycobacterium tuberculosis</i>	CSU#93	[19 31 25 17]	NO DATA	[25 25 34 20]
<i>Mycobacterium tuberculosis</i>	CDC 1551	[19 31 24 18]	NO DATA	[25 25 34 20]
<i>Mycobacterium tuberculosis</i>	H37Rv (lab strain)	[19 31 24 18]	NO DATA	[25 25 34 20]
<i>Mycoplasma pneumoniae</i>	M129	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MRSA252	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MSSA476	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	COL	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	Mu50	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	MW2	NO DATA	NO DATA	NO DATA
<i>Staphylococcus aureus</i>	N315	NO DATA	NO DATA	NO DATA

<i>aureus</i>				
<i>Staphylococcus aureus</i>	NCTC 8325	NO DATA	NO DATA	NO DATA
<i>Streptococcus agalactiae</i>	NEM316	NO DATA	NO DATA	NO DATA
<i>Streptococcus equi</i>	NC 002955	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS8232	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS315	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SSI-1	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	MGAS10394	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	Manfredo (M5)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pyogenes</i>	SF370 (M1)	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	670	NO DATA	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	R6	[20 30 19 23]	NO DATA	NO DATA
<i>Streptococcus pneumoniae</i>	TIGR4	[20 30 19 23]	NO DATA	NO DATA
<i>Streptococcus gordonii</i>	NCTC7868	NO DATA	NO DATA	NO DATA
<i>Streptococcus mitis</i>	NCTC 12261	NO DATA	NO DATA	NO DATA
<i>Streptococcus mutans</i>	UA159	NO DATA	NO DATA	NO DATA

[0121] Four sets of throat samples from military recruits at different military facilities taken at different time points were analyzed using the primers of the present invention. The first set was collected at a military training center from November 1 to December 20, 2002 during one of the most severe outbreaks of pneumonia associated with group A *Streptococcus* in the United States since 1968. During this outbreak, fifty-one throat swabs were taken from both healthy and hospitalized recruits and plated on blood agar for selection of putative group A *Streptococcus* colonies. A second set of 15 original patient specimens was taken during the height of this group A *Streptococcus*-associated respiratory disease outbreak. The third set were historical samples, including twenty-seven isolates of group A *Streptococcus*, from disease outbreaks at this and other military training facilities during previous years. The fourth set of samples was collected from five geographically separated military facilities in the continental U.S. in the winter immediately following the severe November/December 2002 outbreak.

[0122] Pure colonies isolated from group A *Streptococcus*-selective media from all four collection periods were analyzed with the surveillance primer set. All samples showed base compositions that precisely matched the four completely sequenced strains of *Streptococcus pyogenes*. Shown in Figure 4 is a 3D diagram of base composition (axes A, G and C) of bioagent identifying amplicons obtained with primer pair number 14 (a precursor of primer pair

number 348 which targets 16S rRNA). The diagram indicates that the experimentally determined base compositions of the clinical samples closely match the base compositions expected for *Streptococcus pyogenes* and are distinct from the expected base compositions of other organisms.

[0123] In addition to the identification of *Streptococcus pyogenes*, other potentially pathogenic organisms were identified concurrently. Mass spectral analysis of a sample whose nucleic acid was amplified by primer pair number 349 (SEQ ID NOs: 49 and 405) exhibited signals of bioagent identifying amplicons with molecular masses that were found to correspond to analogous base compositions of bioagent identifying amplicons of *Streptococcus pyogenes* (A27 G32 C24 T18), *Neisseria meningitidis* (A25 G27 C22 T18), and *Haemophilus influenzae* (A28 G28 C25 T20) (see Figure 5 and Table 6B). These organisms were present in a ratio of 4:5:20 as determined by comparison of peak heights with peak height of an internal PCR calibration standard as described in commonly owned U. S. Patent Application Serial No: 60/545,425 which is incorporated herein by reference in its entirety.

[0124] Since certain division-wide primers that target housekeeping genes are designed to provide coverage of specific divisions of bacteria to increase the confidence level for identification of bacterial species, they are not expected to yield bioagent identifying amplicons for organisms outside of the specific divisions. For example, primer pair number 356 (SEQ ID NOs: 232:592) primarily amplifies the nucleic acid of members of the classes *Bacilli* and *Clostridia* and is not expected to amplify proteobacteria such as *Neisseria meningitidis* and *Haemophilus influenzae*. As expected, analysis of the mass spectrum of amplification products obtained with primer pair number 356 does not indicate the presence of *Neisseria meningitidis* and *Haemophilus influenzae* but does indicate the presence of *Streptococcus pyogenes* (Figures 3 and 6, Table 6B). Thus, these primers or types of primers can confirm the absence of particular bioagents from a sample.

[0125] The 15 throat swabs from military recruits were found to contain a relatively small set of microbes in high abundance. The most common were *Haemophilus influenza*, *Neisseria meningitidis*, and *Streptococcus pyogenes*. *Staphylococcus epidermidis*, *Moraxella catarrhalis*, *Corynebacterium pseudodiphtheriticum*, and *Staphylococcus aureus* were present in fewer samples. An equal number of samples from healthy volunteers from three different geographic locations, were identically analyzed. Results indicated that the healthy volunteers have bacterial



flora dominated by multiple, commensal non-beta-hemolytic *Streptococcal* species, including the viridans group streptococci (*S. parasanguinis*, *S. vestibularis*, *S. mitis*, *S. oralis* and *S. pneumoniae*; data not shown), and none of the organisms found in the military recruits were found in the healthy controls at concentrations detectable by mass spectrometry. Thus, the military recruits in the midst of a respiratory disease outbreak had a dramatically different microbial population than that experienced by the general population in the absence of epidemic disease.

**[0126] Example 8: Drill-down Analysis for Determination of *emm*-Type of *Streptococcus pyogenes* in Epidemic Surveillance**

**[0127]** As a continuation of the epidemic surveillance investigation of Example 7, determination of sub-species characteristics (genotyping) of *Streptococcus pyogenes*, was carried out based on a strategy that generates strain-specific signatures according to the rationale of Multi-Locus Sequence Typing (MLST). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced (Enright et al. *Infection and Immunity*, 2001, 69, 2416-2427). In classic MLST analysis, internal fragments of several housekeeping genes are amplified and sequenced. In the present investigation, bioagent identifying amplicons from housekeeping genes were produced using drill-down primers and analyzed by mass spectrometry. Since mass spectral analysis results in molecular mass, from which base composition can be determined, the challenge was to determine whether resolution of *emm* classification of strains of *Streptococcus pyogenes* could be determined.

**[0128]** An alignment was constructed of concatenated alleles of seven MLST housekeeping genes (glucose kinase (gki), glutamine transporter protein (gtr), glutamate racemase (murI), DNA mismatch repair protein (mutS), xanthine phosphoribosyl transferase (xpt), and acetyl-CoA acetyl transferase (yqiL)) from each of the 212 previously *emm*-typed strains of *Streptococcus pyogenes*. From this alignment, the number and location of primer pairs that would maximize strain identification via base composition was determined. As a result, 6 primer pairs were chosen as standard drill-down primers for determination of *emm*-type of *Streptococcus pyogenes*. These six primer pairs are displayed in Table 7. This drill-down set comprises primers with T modifications (note TMOD designation in primer names) which constitutes a functional improvement with regard to prevention of non-templated adenylation (*vide supra*) relative to originally selected primers which are displayed below in the same row.

**Table 7: Group A *Streptococcus* Drill-Down Primer Pairs**

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
442	SP101_SPET11_358_387_TM0D_F	311	SP101_SPET11_448_473_TM0D_R	669	gki
80	SP101_SPET11_358_387_F	310	SP101_SPET11_448_473_TM0D_R	668	gki
443	SP101_SPET11_600_629_TM0D_F	314	SP101_SPET11_686_714_TM0D_R	671	gtr
81	SP101_SPET11_600_629_F	313	SP101_SPET11_686_714_R	670	gtr
426	SP101_SPET11_1314_1336_TM0D_F	278	SP101_SPET11_1403_1431_TM0D_R	633	murI
86	SP101_SPET11_1314_1336_F	277	SP101_SPET11_1403_1431_R	632	murI
430	SP101_SPET11_1807_1835_TM0D_F	286	SP101_SPET11_1901_1927_TM0D_R	641	mutS
90	SP101_SPET11_1807_1835_F	285	SP101_SPET11_1901_1927_R	640	mutS
438	SP101_SPET11_3075_3103_TM0D_F	302	SP101_SPET11_3168_3196_TM0D_R	657	xpt
96	SP101_SPET11_3075_3103_F	301	SP101_SPET11_3168_3196_R	656	xpt
441	SP101_SPET11_3511_3535_TM0D_F	309	SP101_SPET11_3605_3629_TM0D_R	664	yqiL
98	SP101_SPET11_3511_3535_F	308	SP101_SPET11_3605_3629_R	663	yqiL

[0129] The primers of Table 7 were used to produce bioagent identifying amplicons from nucleic acid present in the clinical samples. The bioagent identifying amplicons which were subsequently analyzed by mass spectrometry and base compositions corresponding to the molecular masses were calculated.

[0130] Of the 51 samples taken during the peak of the November/December 2002 epidemic (Table 8A-C rows 1-3), all except three samples were found to represent *emm3*, a Group A *Streptococcus* genotype previously associated with high respiratory virulence. The three outliers were from samples obtained from healthy individuals and probably represent non-epidemic strains. Archived samples (Tables 8A-C rows 5-13) from historical collections showed a greater heterogeneity of base compositions and *emm* types as would be expected from different epidemics occurring at different places and dates. The results of the mass spectrometry analysis and *emm* gene sequencing were found to be concordant for the epidemic and historical samples.

**Table 8A: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 426 and 430**

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	murI (Primer Pair No. 426)	mutS (Primer Pair No. 430)
48	3	3	MCRD San Diego (Cultured)	2002	A39 G25 C20 T34	A38 G27 C23 T33
2	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
15	3	ND			A39 G25 C20 T34	A38 G27 C23 T33
6	3	3	NHRC San Diego-Archive (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
3	5,58	5			A40 G24 C20 T34	A38 G27 C23 T33
6	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	11	11			A39 G25 C20 T34	A38 G27 C23 T33
3	12	12			A40 G24 C20 T34	A38 G26 C24 T33
1	22	22			A39 G25 C20 T34	A38 G27 C23 T33
3	25,75	75			A39 G25 C20 T34	A38 G27 C23 T33
4	44/61,82,9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	53,91	91			A39 G25 C20 T34	A38 G27 C23 T33
1	2	2			A39 G25 C20 T34	A38 G27 C24 T32
2	3	3	Ft. Leonard Wood (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
1	4	4			A39 G25 C20 T34	A38 G27 C23 T33
1	6	6			A40 G24 C20 T34	A38 G27 C23 T33
11	25 or 75	75			A39 G25 C20 T34	A38 G27 C23 T33
1	25,75, 33, 34,4,52,84	75			A39 G25 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	44/61			A40 G24 C20 T34	A38 G26 C24 T33
2	5 or 58	5			A40 G24 C20 T34	A38 G27 C23 T33
3	1	1			A40 G24 C20 T34	A38 G27 C23 T33
2	3	3	Ft. Sill (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
1	4	4			A39 G25 C20 T34	A38 G27 C23 T33
1	28	28			A39 G25 C20 T34	A38 G27 C23 T33
1	3	3			A39 G25 C20 T34	A38 G27 C23 T33
1	4	4	Ft. Benning (Cultured)	2003	A39 G25 C20 T34	A38 G27 C23 T33
3	6	6			A40 G24 C20 T34	A38 G27 C23 T33
1	11	11			A39 G25 C20 T34	A38 G27 C23 T33
1	13	94**			A40 G24 C20 T34	A38 G27 C23 T33
1	44/61 or 82 or 9	82			A40 G24 C20 T34	A38 G26 C24 T33
1	5 or 58	58			A40 G24 C20 T34	A38 G27 C23 T33
1	78 or 89	89			A39 G25 C20 T34	A38 G27 C23 T33
2	5 or 58	ND	Lackland AFB (Throat Swabs)	2003	A40 G24 C20 T34	A38 G27 C23 T33
1	2				A39 G25 C20 T34	A38 G27 C24 T32
1	81 or 90				A40 G24 C20 T34	A38 G27 C23 T33
1	78				A38 G26 C20 T34	A38 G27 C23 T33
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A39 G25 C20 T34	A38 G27 C23 T33
1	3	ND			No detection	A38 G27 C23 T33
1	3	ND			No detection	No detection
1	3	ND			No detection	No detection
2	3	ND			No detection	A38 G27 C23 T33
3	No detection	ND			No detection	No detection

**Table 8B: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441**

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	xpt (Primer Pair No. 438)	yqiL (Primer Pair No. 441)
48	3	3	MCRD San Diego (Cultured)	2002	A30 G36 C20 T36	A40 G29 C19 T31
2	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	28	28			A30 G36 C20 T36	A41 G28 C18 T32
15	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
6	3	3	NHRC San Diego-Archive (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
3	5,58	5			A30 G36 C20 T36	A40 G29 C19 T31
6	6	6			A30 G36 C20 T36	A40 G29 C19 T31
1	11	11			A30 G36 C20 T36	A40 G29 C19 T31
3	12	12			A30 G36 C19 T37	A40 G29 C19 T31
1	22	22			A30 G36 C20 T36	A40 G29 C19 T31
3	25,75	75			A30 G36 C20 T36	A40 G29 C19 T31
4	44/61,82,9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	53,91	91			A30 G36 C19 T37	A40 G29 C19 T31
1	2	2			A30 G36 C20 T36	A40 G29 C19 T31
2	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
1	6	6	Ft. Leonard Wood (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
11	25 or 75	75			A30 G36 C20 T36	A40 G29 C19 T31
1	25,75, 33, 34,4,52,84	75			A30 G36 C19 T37	A40 G29 C19 T31
1	44/61 or 82 or 9	44/61			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58	5			A30 G36 C20 T36	A40 G29 C19 T31
3	1	1			A30 G36 C19 T37	A40 G29 C19 T31
2	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
1	28	28	Ft. Sill (Cultured)	2003	A30 G36 C20 T36	A41 G28 C18 T32
1	3	3			A30 G36 C20 T36	A40 G29 C19 T31
1	4	4			A30 G36 C19 T37	A41 G28 C19 T31
1	3	3			A30 G36 C20 T36	A41 G28 C19 T31
1	4	4	Ft. Benning (Cultured)	2003	A30 G36 C20 T36	A40 G29 C19 T31
3	6	6			A30 G36 C19 T37	A41 G28 C19 T31
1	11	11			A30 G36 C20 T36	A40 G29 C19 T31
1	13	94**			A30 G36 C20 T36	A41 G28 C19 T31
1	44/61 or 82 or 9	82			A30 G36 C20 T36	A41 G28 C19 T31
1	5 or 58	58			A30 G36 C20 T36	A40 G29 C19 T31
1	78 or 89	89			A30 G36 C20 T36	A41 G28 C19 T31
2	5 or 58	ND	Lackland AFB (Throat Swabs)	2003	A30 G36 C20 T36	A40 G29 C19 T31
1	2				A30 G36 C20 T36	A40 G29 C19 T31
1	81 or 90				A30 G36 C20 T36	A40 G29 C19 T31
1	78				A30 G36 C20 T36	A41 G28 C19 T31
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
1	3	ND			A30 G36 C20 T36	No detection
1	3	ND			No detection	A40 G29 C19 T31
2	3	ND			A30 G36 C20 T36	A40 G29 C19 T31
3	No detection	ND			No detection	No detection

**Table 8C: Base Composition Analysis of Bioagent Identifying Amplicons of Group A *Streptococcus* samples from Six Military Installations Obtained with Primer Pair Nos. 438 and 441**

# of Instances	emm-type by Mass Spectrometry	emm-Gene Sequencing	Location (sample)	Year	gki (Primer Pair No. 442)	gtr ((Primer Pair No. 443)
48	3	3	MCRD San Diego (Cultured)	2002	A32 G35 C17 T32	A39 G28 C16 T32
2	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
15	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
6	3	3			A32 G35 C17 T32	A39 G28 C16 T32
3	5,58	5	NHRC San Diego-Archive (Cultured)	2003	A30 G36 C20 T30	A39 G28 C15 T33
6	6	6			A31 G35 C17 T33	A39 G28 C15 T33
1	11	11			A30 G36 C20 T30	A39 G28 C16 T32
3	12	12			A31 G35 C17 T33	A39 G28 C15 T33
1	22	22			A31 G35 C17 T33	A38 G29 C15 T33
3	25,75	75			A30 G36 C17 T33	A39 G28 C15 T33
4	44/61,82,9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	53,91	91			A32 G35 C17 T32	A39 G28 C16 T32
1	2	2			A30 G36 C17 T33	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4	Ft. Leonard Wood (Cultured)	2003	A31 G35 C17 T33	A39 G28 C15 T33
1	6	6			A31 G35 C17 T33	A39 G28 C15 T33
11	25 or 75	75			A30 G36 C17 T33	A39 G28 C15 T33
1	25,75, 33, 34,4,52,84	75			A30 G36 C17 T33	A39 G28 C15 T33
1	44/61 or 82 or 9	44/61			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58	5			A30 G36 C20 T30	A39 G28 C15 T33
3	1	1			A30 G36 C18 T32	A39 G28 C15 T33
2	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4	Ft. Sill (Cultured)	2003	A31 G35 C17 T33	A39 G28 C15 T33
1	28	28			A30 G36 C17 T33	A39 G28 C16 T32
1	3	3			A32 G35 C17 T32	A39 G28 C16 T32
1	4	4			A31 G35 C17 T33	A39 G28 C15 T33
3	6	6	Ft. Benning (Cultured)	2003	A31 G35 C17 T33	A39 G28 C15 T33
1	11	11			A30 G36 C20 T30	A39 G28 C16 T32
1	13	94**			A30 G36 C19 T31	A39 G28 C15 T33
1	44/61 or 82 or 9	82			A30 G36 C18 T32	A39 G28 C15 T33
1	5 or 58	58			A30 G36 C20 T30	A39 G28 C15 T33
1	78 or 89	89			A30 G36 C18 T32	A39 G28 C15 T33
2	5 or 58	ND	Lackland AFB (Throat Swabs)	2003	A30 G36 C20 T30	A39 G28 C15 T33
1	2				A30 G36 C17 T33	A39 G28 C15 T33
1	81 or 90				A30 G36 C17 T33	A39 G28 C15 T33
1	78				A30 G36 C18 T32	A39 G28 C15 T33
3***	No detection				No detection	No detection
7	3	ND	MCRD San Diego (Throat Swabs)	2002	A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND			No detection	No detection
1	3	ND			A32 G35 C17 T32	A39 G28 C16 T32
1	3	ND			A32 G35 C17 T32	No detection
2	3	ND			A32 G35 C17 T32	No detection
3	No detection	ND			No detection	No detection

**[0131] Example 9: Design of Calibrant Polynucleotides based on Bioagent Identifying Amplicons for Identification of Species of Bacteria (Bacterial Bioagent Identifying Amplicons)**

**[0132]** This example describes the design of 19 calibrant polynucleotides based on bacterial bioagent identifying amplicons corresponding to the primers of the broad surveillance set (Table 4) and the *Bacillus anthracis* drill-down set (Table 5).

**[0133]** Calibration sequences were designed to simulate bacterial bioagent identifying amplicons produced by the T modified primer pairs shown in Table 4 (primer names have the designation "TMOD"). The calibration sequences were chosen as a representative member of the section of bacterial genome from specific bacterial species which would be amplified by a given primer pair. The model bacterial species upon which the calibration sequences are based are also shown in Table 9. For example, the calibration sequence chosen to correspond to an amplicon produced by primer pair no. 361 is SEQ ID NO: 722. In Table 9, the forward (\_F) or reverse (\_R) primer name indicates the coordinates of an extraction representing a gene of a standard reference bacterial genome to which the primer hybridizes e.g.: the forward primer name 16S\_EC\_713\_732\_TMOD\_F indicates that the forward primer hybridizes to residues 713-732 of the gene encoding 16S ribosomal RNA in an *E. coli* reference sequence (in this case, the reference sequence is an extraction consisting of residues 4033120-4034661 of the genomic sequence of *E. coli* K12 (GenBank gi number 16127994). Additional gene coordinate reference information is shown in Table 10. The designation "TMOD" in the primer names indicates that the 5' end of the primer has been modified with a non-matched template T residue which prevents the PCR polymerase from adding non-templated adenosine residues to the 5' end of the amplification product, an occurrence which may result in miscalculation of base composition from molecular mass data (*vide supra*).

**[0134]** The 19 calibration sequences described in Tables 9 and 10 were combined into a single calibration polynucleotide sequence (SEQ ID NO: 741 - which is herein designated a "combination calibration polynucleotide") which was then cloned into a pCR®-Blunt vector (Invitrogen, Carlsbad, CA). This combination calibration polynucleotide can be used in conjunction with the primers of Table 9 as an internal standard to produce calibration amplicons for use in determination of the quantity of any bacterial bioagent. Thus, for example, when the combination calibration polynucleotide vector is present in an amplification reaction mixture, a calibration amplicon based on primer pair 346 (16S rRNA) will be produced in an amplification

reaction with primer pair 346 and a calibration amplicon based on primer pair 363 (rpoC) will be produced with primer pair 363. Coordinates of each of the 19 calibration sequences within the calibration polynucleotide (SEQ ID NO: 783) are indicated in Table 10.

**Table 9: Bacterial Primer Pairs for Production of Bacterial Bioagent Identifying Amplicons and Corresponding Representative Calibration Sequences**

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Calibration Sequence Model Species	Calibration Sequence (SEQ ID NO:)
361	16S_EC_1090_1111_2_TMOD_F	5	16S_EC_1175_1196_TMOD_R	370	<i>Bacillus anthracis</i>	764
346	16S_EC_713_732_TMOD_F	27	16S_EC_789_809_TMOD_R	389	<i>Bacillus anthracis</i>	765
347	16S_EC_785_806_TMOD_F	30	16S_EC_880_897_TMOD_R	392	<i>Bacillus anthracis</i>	766
348	16S_EC_960_981_TMOD_F	38	16S_EC_1054_1073_TMOD_R	363	<i>Bacillus anthracis</i>	767
349	23S_EC_1826_1843_TMOD_F	49	23S_EC_1906_1924_TMOD_R	405	<i>Bacillus anthracis</i>	768
360	23S_EC_2646_2667_TMOD_F	60	23S_EC_2745_2765_TMOD_R	416	<i>Bacillus anthracis</i>	769
350	CAPC_BA_274_303_TMOD_F	98	CAPC_BA_349_376_TMOD_R	452	<i>Bacillus anthracis</i>	770
351	CYA_BA_1353_1379_TMOD_F	128	CYA_BA_1448_1467_TMOD_R	483	<i>Bacillus anthracis</i>	771
352	INFB_EC_1365_1393_TMOD_F	161	INFB_EC_1439_1467_TMOD_R	516	<i>Bacillus anthracis</i>	772
353	LEF_BA_756_781_TMOD_F	175	LEF_BA_843_872_TMOD_R	531	<i>Bacillus anthracis</i>	773
356	RPLB_EC_650_679_TMOD_F	232	RPLB_EC_739_762_TMOD_R	592	<i>Clostridium botulinum</i>	774
449	RPLB_EC_690_710_F	237	RPLB_EC_737_758_R	589	<i>Clostridium botulinum</i>	775
359	RPOB_EC_1845_1866_TMOD_F	241	RPOB_EC_1909_1929_TMOD_R	597	<i>Yersinia Pestis</i>	776
362	RPOB_EC_3799_3821_TMOD_F	245	RPOB_EC_3862_3888_TMOD_R	603	<i>Burkholderia mallei</i>	777
363	RPOC_EC_2146_2174_TMOD_F	257	RPOC_EC_2227_2245_TMOD_R	621	<i>Burkholderia mallei</i>	778
354	RPOC_EC_2218_2241_TMOD_F	262	RPOC_EC_2313_2337_TMOD_R	625	<i>Bacillus anthracis</i>	779
355	SSPE_BA_115_137_TMOD_F	321	SSPE_BA_197_222_TMOD_R	687	<i>Bacillus anthracis</i>	780
367	TUFB_EC_957_979_TMOD_F	345	TUFB_EC_1034_1058_TMOD_R	701	<i>Burkholderia mallei</i>	781
358	VALS_EC_1105_1124_TMOD_F	350	VALS_EC_1195_1218_TMOD_R	712	<i>Yersinia Pestis</i>	782

**Table 10: Primer Pair Gene Coordinate References and Calibration Polynucleotide Sequence Coordinates within the Combination Calibration Polynucleotide**

Bacterial Gene and Species	Gene Extraction Coordinates of Genomic or Plasmid Sequence	Reference GenBank GI No. of Genomic (G) or Plasmid (P) Sequence	Primer Pair No.	Coordinates of Calibration Sequence in Combination Calibration Polynucleotide (SEQ ID NO: 783)
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	346	16..109
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	347	83..190
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	348	246..353
16S <i>E. coli</i>	4033120..4034661	16127994 (G)	361	368..469
23S <i>E. coli</i>	4166220..4169123	16127994 (G)	349	743..837
23S <i>E. coli</i>	4166220..4169123	16127994 (G)	360	865..981
rpoB <i>E. coli</i>	4178823..4182851 (complement strand)	16127994 (G)	359	1591..1672
rpoB <i>E. coli</i>	4178823..4182851 (complement strand)	16127994 (G)	362	2081..2167
rpoC <i>E. coli</i>	4182928..4187151	16127994 (G)	354	1810..1926
rpoC <i>E. coli</i>	4182928..4187151	16127994 (G)	363	2183..2279
infB <i>E. coli</i>	3313655..3310983 (complement strand)	16127994 (G)	352	1692..1791
tufB <i>E. coli</i>	4173523..4174707	16127994 (G)	367	2400..2498
rplB <i>E. coli</i>	3449001..3448180	16127994 (G)	356	1945..2060
rplB <i>E. coli</i>	3449001..3448180	16127994 (G)	449	1986..2055
vals <i>E. coli</i>	4481405..4478550 (complement strand)	16127994 (G)	358	1462..1572

capC <i>B. anthracis</i>	56074..55628 (complement strand)	6470151 (P)	350	2517..2616
cya <i>B. anthracis</i>	156626..154288 (complement strand)	4894216 (P)	351	1338..1449
lef <i>B. anthracis</i>	127442..129921	4894216 (P)	353	1121..1234
sspE <i>B. anthracis</i>	226496..226783	30253828 (G)	355	1007-1104

**[0135] Example 10: Use of a Calibration Polynucleotide for Determining the Quantity of *Bacillus Anthracis* in a Sample Containing a Mixture of Microbes**

**[0136]** The process described in this example is shown in Figure 7. The capC gene is a gene involved in capsule synthesis which resides on the pX02 plasmid of *Bacillus anthracis*. Primer pair number 350 (see Tables 9 and 10) was designed to identify *Bacillus anthracis* via production of a bacterial bioagent identifying amplicon. Known quantities of the combination calibration polynucleotide vector described in Example 3 were added to amplification mixtures containing bacterial bioagent nucleic acid from a mixture of microbes which included the Ames strain of *Bacillus anthracis*. Upon amplification of the bacterial bioagent nucleic acid and the combination calibration polynucleotide vector with primer pair no. 350, bacterial bioagent identifying amplicons and calibration amplicons were obtained and characterized by mass spectrometry. A mass spectrum measured for the amplification reaction is shown in Figure 8). The molecular masses of the bioagent identifying amplicons provided the means for identification of the bioagent from which they were obtained (Ames strain of *Bacillus anthracis*) and the molecular masses of the calibration amplicons provided the means for their identification as well. The relationship between the abundance (peak height) of the calibration amplicon signals and the bacterial bioagent identifying amplicon signals provides the means of calculation of the copies of the pX02 plasmid of the Ames strain of *Bacillus anthracis*. Methods of calculating quantities of molecules based on internal calibration procedures are well known to those of ordinary skill in the art.

**[0137]** Averaging the results of 10 repetitions of the experiment described above, enabled a calculation that indicated that the quantity of Ames strain of *Bacillus anthracis* present in the sample corresponds to approximately 10 copies of pX02 plasmid.

**[0138] Example 11: Drill-down Genotyping of *Campylobacter* Species**

**[0139]** A series of drill-down primers were designed as described in Example 1 with the objective of identification of different strains of *Campylobacter jejuni*. The primers are listed in Table 11 with the designation "CJST\_CJ." Housekeeping genes to which the primers hybridize and produce bioagent identifying amplicons include: tkt (transketolase), glyA (serine



hydroxymethyltransferase), *glbA* (citrate synthase), *aspA* (aspartate ammonia lyase), *glnA* (glutamine synthase), *pgm* (phosphoglycerate mutase), and *uncA* (ATP synthetase alpha chain).

**Table 11: *Campylobacter* Drill-down Primer Pairs**

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)	Target Gene
1053	CJST CJ 1080 1110 F	102	CJST CJ 1166 1198 R	456	<i>glbA</i>
1064	CJST CJ 1680 1713 F	107	CJST CJ 1795 1822 R	461	<i>glyA</i>
1054	CJST CJ 2060 2090 F	109	CJST CJ 2148 2174 R	463	<i>pgm</i>
1049	CJST CJ 2636 2668 F	113	CJST CJ 2753 2777 R	467	<i>tkt</i>
1048	CJST CJ 360 394 F	119	CJST CJ 442 476 R	472	<i>aspA</i>
1047	CJST CJ 584 616 F	121	CJST CJ 663 692 R	474	<i>glnA</i>

[0140] The primers were used to amplify nucleic acid from 50 food product samples provided by the USDA, 25 of which contained *Campylobacter jejuni* and 25 of which contained *Campylobacter coli*. Primers used in this study were developed primarily for the discrimination of *Campylobacter jejuni* clonal complexes and for distinguishing *Campylobacter jejuni* from *Campylobacter coli*. Finer discrimination between *Campylobacter coli* types is also possible by using specific primers targeted to loci where closely-related *Campylobacter coli* isolates demonstrate polymorphisms between strains. The conclusions of the comparison of base composition analysis with sequence analysis are shown in Tables 12A-C.

**Table 12A — Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1048 and 1047**

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1048 ( <i>aspA</i> )	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1047 ( <i>glnA</i> )
J-1	<i>C. jejuni</i>	Goose	ST 690 /692/707/991	ST 991	RM3673	A30 G25 C16 T46	A47 G21 C16 T25
J-2	<i>C. jejuni</i>	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A30 G25 C16 T46	A48 G21 C17 T23
J-3	<i>C. jejuni</i>	Human	Complex 354/179	ST 436	RM4194	A30 G25 C15 T47	A48 G21 C18 T22
J-4	<i>C. jejuni</i>	Human	Complex 257	ST 257, complex 257	RM4197	A30 G25 C16 T46	A48 G21 C18 T22
J-5	<i>C. jejuni</i>	Human	Complex 52	ST 52, complex 52	RM4277	A30 G25 C16 T46	A48 G21 C17 T23
J-6	<i>C. jejuni</i>	Human	Complex 443	ST 51, complex 443	RM4275	A30 G25 C15 T47	A48 G21 C17 T23
					RM4279	A30 G25 C15 T47	A48 G21 C17 T23
J-7	<i>C. jejuni</i>	Human	Complex 42	ST 604, complex 42	RM1864	A30 G25 C15 T47	A48 G21 C18 T22
J-8	<i>C. jejuni</i>	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A30 G25 C15 T47	A48 G21 C18 T22
J-9	<i>C. jejuni</i>	Human	Complex 45/283	ST 147, Complex 45	RM3203	A30 G25 C15 T47	A47 G21 C18 T23
	<i>C. jejuni</i>	Human	Consistent	ST 828	RM4183	A31 G27 C20 T39	A48 G21 C16 T24

C-1	C. coli	Poultry	with 74 closely related sequence types (none belong to a clonal complex)	ST 832	RM1169	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1056	RM1857	A31 G27 C20 T39	A48 G21 C16 T24
				ST 889	RM1166	A31 G27 C20 T39	A48 G21 C16 T24
				ST 829	RM1182	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1050	RM1518	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1051	RM1521	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1053	RM1523	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1055	RM1527	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1017	RM1529	A31 G27 C20 T39	A48 G21 C16 T24
				ST 860	RM1840	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1063	RM2219	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1066	RM2241	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1067	RM2243	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1068	RM2439	A31 G27 C20 T39	A48 G21 C16 T24
		Swine		ST 1016	RM3230	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1069	RM3231	A31 G27 C20 T39	A48 G21 C16 T24
		Unknown		ST 1061	RM1904	A31 G27 C20 T39	A48 G21 C16 T24
				ST 825	RM1534	A31 G27 C20 T39	A48 G21 C16 T24
			ST 901	RM1505	A31 G27 C20 T39	A48 G21 C16 T24	
C-2	C. coli	Human	ST 895	ST 895	RM1532	A31 G27 C19 T40	A48 G21 C16 T24
C-3	C. coli	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1082	RM1178	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1054	RM1525	A31 G27 C20 T39	A48 G21 C16 T24
				ST 1049	RM1517	A31 G27 C20 T39	A48 G21 C16 T24
		Marmoset		ST 891	RM1531	A31 G27 C20 T39	A48 G21 C16 T24

**Table 12B – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1053 and 1064**

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1053 (gltA)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1064 (glyA)
J-1	<i>C. jejuni</i>	Goose	ST 690 /692/707/991	ST 991	RM3673	A24 G25 C23 T47	A40 G29 C29 T45
J-2	<i>C. jejuni</i>	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A24 G25 C23 T47	A40 G29 C29 T45
J-3	<i>C. jejuni</i>	Human	Complex 354/179	ST 436	RM4194	A24 G25 C23 T47	A40 G29 C29 T45
J-4	<i>C. jejuni</i>	Human	Complex 257	ST 257, complex 257	RM4197	A24 G25 C23 T47	A40 G29 C29 T45
J-5	<i>C. jejuni</i>	Human	Complex 52	ST 52, complex 52	RM4277	A24 G25 C23 T47	A39 G30 C26 T48
J-6	<i>C. jejuni</i>	Human	Complex 443	ST 51, complex 443	RM4275	A24 G25 C23 T47	A39 G30 C28 T46
					RM4279	A24 G25 C23 T47	A39 G30 C28 T46
J-7	<i>C. jejuni</i>	Human	Complex 42	ST 604, complex 42	RM1864	A24 G25 C23 T47	A39 G30 C26 T48

J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A24 G25 C23 T47	A38 G31 C28 T46
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A24 G25 C23 T47	A38 G31 C28 T46
	C. jejuni	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A23 G24 C26 T46	A39 G30 C27 T47
C-1	C. coli			ST 832	RM1169	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1056	RM1857	A23 G24 C26 T46	A39 G30 C27 T47
		Poultry		ST 889	RM1166	A23 G24 C26 T46	A39 G30 C27 T47
				ST 829	RM1182	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1050	RM1518	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1051	RM1521	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1053	RM1523	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1055	RM1527	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1017	RM1529	A23 G24 C26 T46	A39 G30 C27 T47
				ST 860	RM1840	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1063	RM2219	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1066	RM2241	A23 G24 C26 T46	A39 G30 C27 T47
		ST 1067		RM2243	A23 G24 C26 T46	A39 G30 C27 T47	
		ST 1068		RM2439	A23 G24 C26 T46	A39 G30 C27 T47	
		Swine		ST 1016	RM3230	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1069	RM3231	A23 G24 C26 T46	NO DATA
				ST 1061	RM1904	A23 G24 C26 T46	A39 G30 C27 T47
Unknown	ST 825	RM1534		A23 G24 C26 T46	A39 G30 C27 T47		
	ST 901	RM1505	A23 G24 C26 T46	A39 G30 C27 T47			
C-2	C. coli	Human	ST 895	ST 895	RM1532	A23 G24 C26 T46	A39 G30 C27 T47
C-3	C. coli	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1082	RM1178	A23 G24 C26 T46	A39 G30 C27 T47
				ST 1054	RM1525	A23 G24 C25 T47	A39 G30 C27 T47
				ST 1049	RM1517	A23 G24 C26 T46	A39 G30 C27 T47
		Marmoset		ST 891	RM1531	A23 G24 C26 T46	A39 G30 C27 T47

**Table 12C – Results of Base Composition Analysis of 50 *Campylobacter* Samples with Drill-down MLST Primer Pair Nos: 1054 and 1049**

Group	Species	Isolate origin	MLST type or Clonal Complex by Base Composition analysis	MLST Type or Clonal Complex by Sequence analysis	Strain	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1054 (pgm)	Base Composition of Bioagent Identifying Amplicon Obtained with Primer Pair No: 1049 (tkk)
J-1	<i>C. jejuni</i>	Goose	ST 690 /692/707/991	ST 991	RM3673	A26 G33 C18 T38	A41 G28 C35 T38
J-2	<i>C. jejuni</i>	Human	Complex 206/48/353	ST 356, complex 353	RM4192	A26 G33 C19 T37	A41 G28 C36 T37
J-3	<i>C. jejuni</i>	Human	Complex 354/179	ST 436	RM4194	A27 G32 C19 T37	A42 G28 C36 T36
J-4	<i>C. jejuni</i>	Human	Complex 257	ST 257, complex 257	RM4197	A27 G32 C19 T37	A41 G29 C35 T37
J-5	<i>C. jejuni</i>	Human	Complex 52	ST 52, complex 52	RM4277	A26 G33 C18 T38	A41 G28 C36 T37

J-6	C. jejuni	Human	Complex 443	ST 51, complex 443	RM4275	A27 G31 C19 T38	A41 G28 C36 T37
					RM4279	A27 G31 C19 T38	A41 G28 C36 T37
J-7	C. jejuni	Human	Complex 42	ST 604, complex 42	RM1864	A27 G32 C19 T37	A42 G28 C35 T37
J-8	C. jejuni	Human	Complex 42/49/362	ST 362, complex 362	RM3193	A26 G33 C19 T37	A42 G28 C35 T37
J-9	C. jejuni	Human	Complex 45/283	ST 147, Complex 45	RM3203	A28 G31 C19 T37	A43 G28 C36 T35
	C. jejuni	Human	Consistent with 74 closely related sequence types (none belong to a clonal complex)	ST 828	RM4183	A27 G30 C19 T39	A46 G28 C32 T36
C-1	C. coli			ST 832	RM1169	A27 G30 C19 T39	A46 G28 C32 T36
		ST 1056		RM1857	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 889		RM1166	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 829		RM1182	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1050		RM1518	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1051		RM1521	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1053		RM1523	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1055		RM1527	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1017		RM1529	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 860		RM1840	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1063		RM2219	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1066		RM2241	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1067		RM2243	A27 G30 C19 T39	A46 G28 C32 T36	
		ST 1068		RM2439	A27 G30 C19 T39	A46 G28 C32 T36	
		Swine		ST 1016	RM3230	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1069	RM3231	A27 G30 C19 T39	A46 G28 C32 T36
				ST 1061	RM1904	A27 G30 C19 T39	A46 G28 C32 T36
				Unknown	ST 825	RM1534	A27 G30 C19 T39
ST 901	RM1505	A27 G30 C19 T39			A46 G28 C32 T36		
C-2	C. coli	Human	ST 895	ST 895	RM1532	A27 G30 C19 T39	A45 G29 C32 T36
C-3	C. coli	Poultry	Consistent with 63 closely related sequence types (none belong to a clonal complex)	ST 1064	RM2223	A27 G30 C19 T39	A45 G29 C32 T36
				ST 1082	RM1178	A27 G30 C19 T39	A45 G29 C32 T36
				ST 1054	RM1525	A27 G30 C19 T39	A45 G29 C32 T36
				ST 1049	RM1517	A27 G30 C19 T39	A45 G29 C32 T36
		Marmoset		ST 891	RM1531	A27 G30 C19 T39	A45 G29 C32 T36

[0141] The base composition analysis method was successful in identification of 12 different strain groups. *Campylobacter jejuni* and *Campylobacter coli* are generally differentiated by all loci. Ten clearly differentiated *Campylobacter jejuni* isolates and 2 major *Campylobacter coli* groups were identified even though the primers were designed for strain typing of

*Campylobacter jejuni*. One isolate (RM4183) which was designated as *Campylobacter jejuni* was found to group with *Campylobacter coli* and also appears to actually be *Campylobacter coli* by full MLST sequencing.

**[0142] Example 12: Identification of *Acinetobacter baumannii* Using Broad Range Survey and Division-Wide Primers in Epidemiological Surveillance**

**[0143]** To test the capability of the broad range survey and division-wide primer sets of Table 4 in identification of *Acinetobacter* species, 183 clinical samples were obtained from individuals participating in, or in contact with individuals participating in Operation Iraqi Freedom (including US service personnel, US civilian patients at the Walter Reed Army Institute of Research (WRAIR), medical staff, Iraqi civilians and enemy prisoners). In addition, 34 environmental samples were obtained from hospitals in Iraq, Kuwait, Germany, the United States and the USNS Comfort, a hospital ship.

**[0144]** Upon amplification of nucleic acid obtained from the clinical samples, primer pairs 346-349, 360, 361, 354, 362 and 363 (Table 4) all produced bacterial bioagent amplicons which identified *Acinetobacter baumannii* in 215 of 217 samples. The organism *Klebsiella pneumoniae* was identified in the remaining two samples. In addition, 14 different strain types (containing single nucleotide polymorphisms relative to a reference strain of *Acinetobacter baumannii*) were identified and assigned arbitrary numbers from 1 to 14. Strain type 1 was found in 134 of the sample isolates and strains 3 and 7 were found in 46 and 9 of the isolates respectively.

**[0145]** The epidemiology of strain type 7 of *Acinetobacter baumannii* was investigated. Strain 7 was found in 4 patients and 5 environmental samples (from field hospitals in Iraq and Kuwait). The index patient infected with strain 7 was a pre-war patient who had a traumatic amputation in March of 2003 and was treated at a Kuwaiti hospital. The patient was subsequently transferred to a hospital in Germany and then to WRAIR. Two other patients from Kuwait infected with strain 7 were found to be non-infectious and were not further monitored. The fourth patient was diagnosed with a strain 7 infection in September of 2003 at WRAIR. Since the fourth patient was not related involved in Operation Iraqi Freedom, it was inferred that the fourth patient was the subject of a nosocomial infection acquired at WRAIR as a result of the spread of strain 7 from the index patient.

[0146] The epidemiology of strain type 3 of *Acinetobacter baumannii* was also investigated. Strain type 3 was found in 46 samples, all of which were from patients (US service members, Iraqi civilians and enemy prisoners) who were treated on the USNS Comfort hospital ship and subsequently returned to Iraq or Kuwait. The occurrence of strain type 3 in a single locale may provide evidence that at least some of the infections at that locale were a result of a nosocomial infections.

[0147] This example thus illustrates an embodiment of the present invention wherein the methods of analysis of bacterial bioagent identifying amplicons provide the means for epidemiological surveillance.

**[0148] Example 13: Selection and Use of MLST *Acinetobacter baumannii* Drill-down Primers**

[0149] To combine the power of high-throughput mass spectrometric analysis of bioagent identifying amplicons with the sub-species characteristic resolving power provided by multi-locus sequence typing (MLST) such as the MLST methods of the MLST Databases at the Max-Planck Institute for Infectious Biology ([web.mpiib-berlin.mpg.de/mlst/dbs/Mcatarrhalis/documents/primersCatarrhalis\\_.html](http://web.mpiib-berlin.mpg.de/mlst/dbs/Mcatarrhalis/documents/primersCatarrhalis_.html)), an additional 21 primer pairs were selected based on analysis of housekeeping genes of the genus *Acinetobacter*. Genes to which the drill-down MLST analogue primers hybridize for production of bacterial bioagent identifying amplicons include anthranilate synthase component I (trpE), adenylate kinase (adk), adenine glycosylase (mutY), fumarate hydratase (fumC), and pyrophosphate phospho-hydratase (ppa). These 21 primer pairs are indicated with reference to sequence listings in Table 13. Primer pair numbers 1151-1154 hybridize to and amplify segments of trpE. Primer pair numbers 1155-1157 hybridize to and amplify segments of adk. Primer pair numbers 1158-1164 hybridize to and amplify segments of mutY. Primer pair numbers 1165-1170 hybridize to and amplify segments of fumC. Primer pair number 1171 hybridizes to and amplifies a segment of ppa. The primer names given in Table 13 indicates the coordinates to which the primers hybridize to a reference sequence which comprises a concatenation of the genes TrpE, efp (elongation factor p), adk, mutT, fumC, and ppa. For example, the forward primer of primer pair 1151 is named AB\_MLST-11-OIF007\_62\_91\_F because it hybridizes to the *Acinetobacter* MLST primer reference sequence of strain type 11 in sample 007 of Operation Iraqi Freedom (OIF) at positions 62 to 91.

**Table 13: MLST Drill-Down Primers for Identification of Sub-species characteristics  
(Strain Type) of Members of the Bacterial Genus *Acinetobacter***

Primer Pair No.	Forward Primer Name	Forward Primer (SEQ ID NO:)	Reverse Primer Name	Reverse Primer (SEQ ID NO:)
1151	AB_MLST-11-OIF007_62_91_F	83	AB_MLST-11-OIF007_169_203_R	426
1152	AB_MLST-11-OIF007_185_214_F	76	AB_MLST-11-OIF007_291_324_R	432
1153	AB_MLST-11-OIF007_260_289_F	79	AB_MLST-11-OIF007_364_393_R	434
1154	AB_MLST-11-OIF007_206_239_F	78	AB_MLST-11-OIF007_318_344_R	433
1155	AB_MLST-11-OIF007_522_552_F	80	AB_MLST-11-OIF007_587_610_R	435
1156	AB_MLST-11-OIF007_547_571_F	81	AB_MLST-11-OIF007_656_686_R	436
1157	AB_MLST-11-OIF007_601_627_F	82	AB_MLST-11-OIF007_710_736_R	437
1158	AB_MLST-11- OIF007_1202_1225_F	65	AB_MLST-11-OIF007_1266_1296_R	420
1159	AB_MLST-11- OIF007_1202_1225_F	65	AB_MLST-11-OIF007_1299_1316_R	421
1160	AB_MLST-11- OIF007_1234_1264_F	66	AB_MLST-11-OIF007_1335_1362_R	422
1161	AB_MLST-11- OIF007_1327_1356_F	67	AB_MLST-11-OIF007_1422_1448_R	423
1162	AB_MLST-11- OIF007_1345_1369_F	68	AB_MLST-11-OIF007_1470_1494_R	424
1163	AB_MLST-11- OIF007_1351_1375_F	69	AB_MLST-11-OIF007_1470_1494_R	424
1164	AB_MLST-11- OIF007_1387_1412_F	70	AB_MLST-11-OIF007_1470_1494_R	424
1165	AB_MLST-11- OIF007_1542_1569_F	71	AB_MLST-11-OIF007_1656_1680_R	425
1166	AB_MLST-11- OIF007_1566_1593_F	72	AB_MLST-11-OIF007_1656_1680_R	425
1167	AB_MLST-11- OIF007_1611_1638_F	73	AB_MLST-11-OIF007_1731_1757_R	427
1168	AB_MLST-11- OIF007_1726_1752_F	74	AB_MLST-11-OIF007_1790_1821_R	428
1169	AB_MLST-11- OIF007_1792_1826_F	75	AB_MLST-11-OIF007_1876_1909_R	429
1170	AB_MLST-11- OIF007_1792_1826_F	75	AB_MLST-11-OIF007_1895_1927_R	430
1171	AB_MLST-11-	77	AB_MLST-11-OIF007_2097_2118_R	431

	OIF007 1970 2002 F			
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[0150] Analysis of bioagent identifying amplicons obtained using the primers of Table 13 for over 200 samples from Operation Iraqi Freedom resulted in the identification of 50 distinct strain type clusters. The largest cluster, designated strain type 11 (ST11) includes 42 sample isolates, all of which were obtained from US service personnel and Iraqi civilians treated at the 28<sup>th</sup> Combat Support Hospital in Baghdad. Several of these individuals were also treated on the hospital ship USNS Comfort. These observations are indicative of significant epidemiological correlation/linkage.

[0151] All of the sample isolates were tested against a broad panel of antibiotics to characterize their antibiotic resistance profiles. As an example of a representative result from antibiotic susceptibility testing, ST11 was found to consist of four different clusters of isolates, each with a varying degree of sensitivity/resistance to the various antibiotics tested which included penicillins, extended spectrum penicillins, cephalosporins, carbipenem, protein synthesis inhibitors, nucleic acid synthesis inhibitors, anti-metabolites, and anti-cell membrane antibiotics. Thus, the genotyping power of bacterial bioagent identifying amplicons, particularly drill-down bacterial bioagent identifying amplicons, has the potential to increase the understanding of the transmission of infections in combat casualties, to identify the source of infection in the environment, to track hospital transmission of nosocomial infections, and to rapidly characterize drug-resistance profiles which enable development of effective infection control measures on a time-scale previously not achievable.

[0152] Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims. Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, internet web sites, and the like) cited in the present application is incorporated herein by reference in its entirety.



**WHAT IS CLAIMED IS:**

1. An oligonucleotide primer selected from the group consisting of: an oligonucleotide primer 16 to 35 nucleobases in length comprising 80% to 100% sequence identity with SEQ ID NO: 26, an oligonucleotide primer 20 to 27 nucleobases in length comprising at least a 20 nucleobase portion of SEQ ID NO: 388, an oligonucleotide primer 22 to 35 nucleobases in length comprising SEQ ID NO: 29, an oligonucleotide primer 18 to 35 nucleobases in length comprising SEQ ID NO: 391, an oligonucleotide primer 22 to 26 nucleobases in length comprising SEQ ID NO: 37, an oligonucleotide primer 20 to 30 nucleobases in length comprising SEQ ID NO: 362, an oligonucleotide primer 13 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 48, an oligonucleotide primer 19 to 35 nucleobases in length comprising SEQ ID NO: 404, an oligonucleotide primer 21 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 160, an oligonucleotide primer 21 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 515, an oligonucleotide primer 17 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 261, an oligonucleotide primer 18 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 624, an oligonucleotide primer 21 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 231, an oligonucleotide primer 17 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 591; an oligonucleotide primer 14 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 349, an oligonucleotide primer 17 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 711, an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 240, an oligonucleotide primer 15 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 596, an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 58, an oligonucleotide primer 21 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 414, an oligonucleotide primer 16 to 35 nucleobases in length comprising at least a 16 nucleobase portion of SEQ ID NO: 369, an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 246, an oligonucleotide primer 19 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 602, an oligonucleotide primer 21 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 256, an oligonucleotide primer 14 to 35 nucleobases in length

comprising 70% to 100% sequence identity with SEQ ID NO: 620, an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 344, an oligonucleotide primer 18 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 700, an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 235, an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 587;

wherein said primer comprises a non-templated T residue on the 5'-end, or at least one non-template tag.

2. A composition comprising one or more of the oligonucleotide primers of claim 1.
3. A composition comprising two or more of the oligonucleotide primers of claim 1.
4. The composition of claim 3 wherein either or both of said oligonucleotide primers comprises at least one modified nucleobase.
5. The composition of claim 3 wherein either or both of said oligonucleotide primers comprises a non-templated T residue on the 5'-end.
6. The composition of claim 3 wherein either or both of said oligonucleotide primers comprises at least one non-template tag.
7. The composition of claim 3 wherein either or both of said oligonucleotide primers comprises at least one molecular mass modifying tag.
8. An oligonucleotide primer selected from the group consisting of: an oligonucleotide primer 16 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 322, and an oligonucleotide primer 19 to 35 nucleobases in length comprising 70% to 100% sequence identity with SEQ ID NO: 686.
9. A composition comprising one or both of the oligonucleotide primers of claim 8.
10. The composition of claim 9 wherein either or both of said oligonucleotide primers comprises at least one modified nucleobase.

11. The composition of claim 9 wherein either or both of said oligonucleotide primers comprises a non-templated T residue on the 5'-end.
12. The composition of claim 9 wherein either or both of said oligonucleotide primers comprises at least one non-template tag.
13. The composition of claim 9 wherein either or both of said oligonucleotide primers comprises at least one molecular mass modifying tag.
14. A kit comprising the composition of claim 3 or claim 9.
15. The kit of claim 14 further comprising at least one calibration polynucleotide.
16. The kit of claim 14 further comprising at least one ion exchange resin linked to magnetic beads.
17. A method for identification of an unknown bacterium comprising:
  - amplifying nucleic acid from said bacterium using the composition of claim 3 or claim 9 to obtain an amplification product;
  - determining the molecular mass of said amplification product;
  - optionally determining the base composition of said amplification product from said molecular mass; and
  - comparing said molecular mass or base composition of said amplification product with a plurality of molecular masses or base compositions of known bacterial bioagent identifying amplicons, wherein a match between said molecular mass or base composition of said amplification product and the molecular mass or base composition of a member of said plurality of molecular masses or base compositions identifies said unknown bacterium.
18. The method of claim 17 wherein said molecular mass is determined by mass spectrometry.
19. A method of determining the presence or absence of a bacterium of a particular clade, genus, species, or sub-species in a sample comprising:

amplifying nucleic acid from said sample using the composition of claim 3 or claim 9 to obtain an amplification product;

determining the molecular mass of said amplification product;

optionally determining the base composition of said amplification product from said molecular mass; and

comparing said molecular mass or base composition of said amplification product with the known molecular masses or base compositions of one or more known clade, genus, species, or sub-species bioagent identifying amplicons, wherein a match between said molecular mass or base composition of said amplification product and the molecular mass or base composition of one or more known clade, genus, species, or sub-species bioagent identifying amplicons indicates the presence of said clade, genus, species, or sub-species in said sample.

20. The method of claim 19 wherein said molecular mass is determined by mass spectrometry.

21. A method for determination of the quantity of an unknown bacterium in a sample comprising:

contacting said sample with the composition of claim 3 or claim 9 and a known quantity of a calibration polynucleotide comprising a calibration sequence;

concurrently amplifying nucleic acid from said bacterium in said sample with the composition of claim 3 or claim 9 and amplifying nucleic acid from said calibration polynucleotide in said sample with the composition of claim 3 or claim 9 to obtain a first amplification product comprising a bacterial bioagent identifying amplicon and a second amplification product comprising a calibration amplicon;

determining the molecular mass and abundance for said bacterial bioagent identifying amplicon and said calibration amplicon; and

distinguishing said bacterial bioagent identifying amplicon from said calibration amplicon based on molecular mass, wherein comparison of bacterial bioagent identifying amplicon abundance and calibration amplicon abundance indicates the quantity of bacterium in said sample.

22. The method of claim 21 further comprising determining the base composition of said bacterial bioagent identifying amplicon.

### Figure 1

1/8

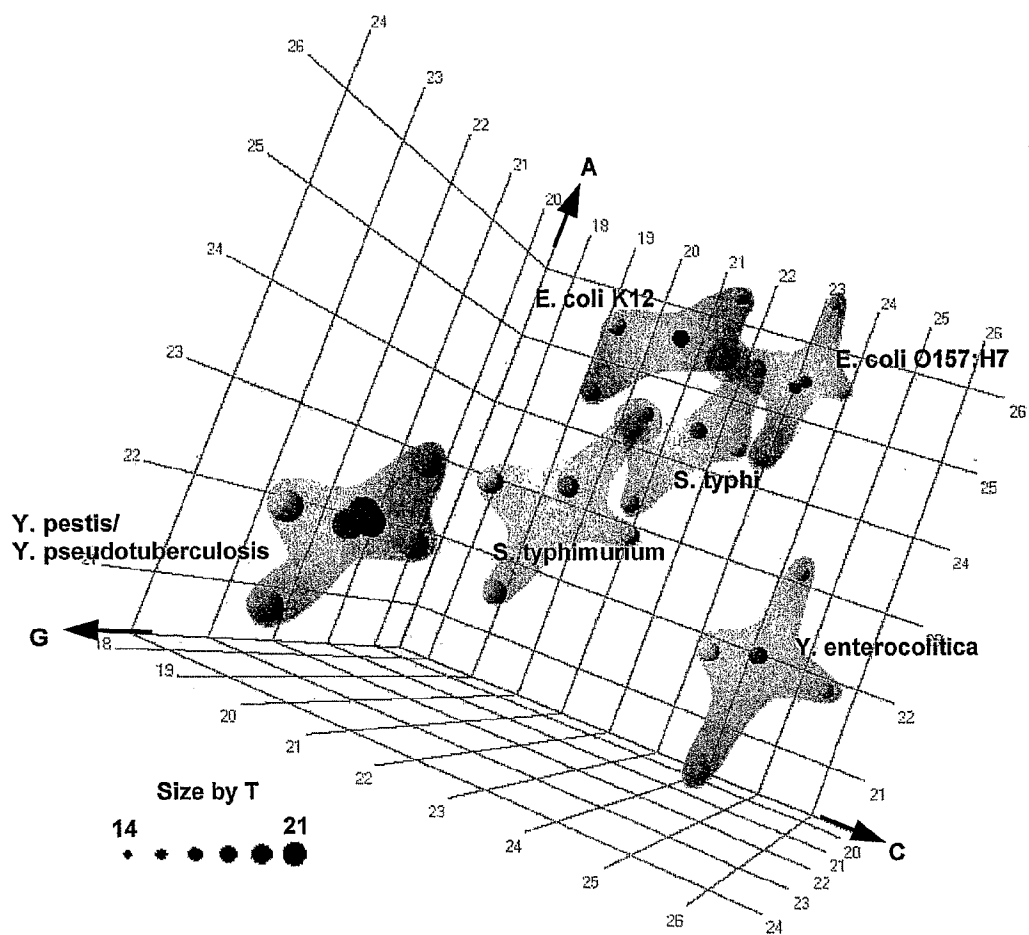


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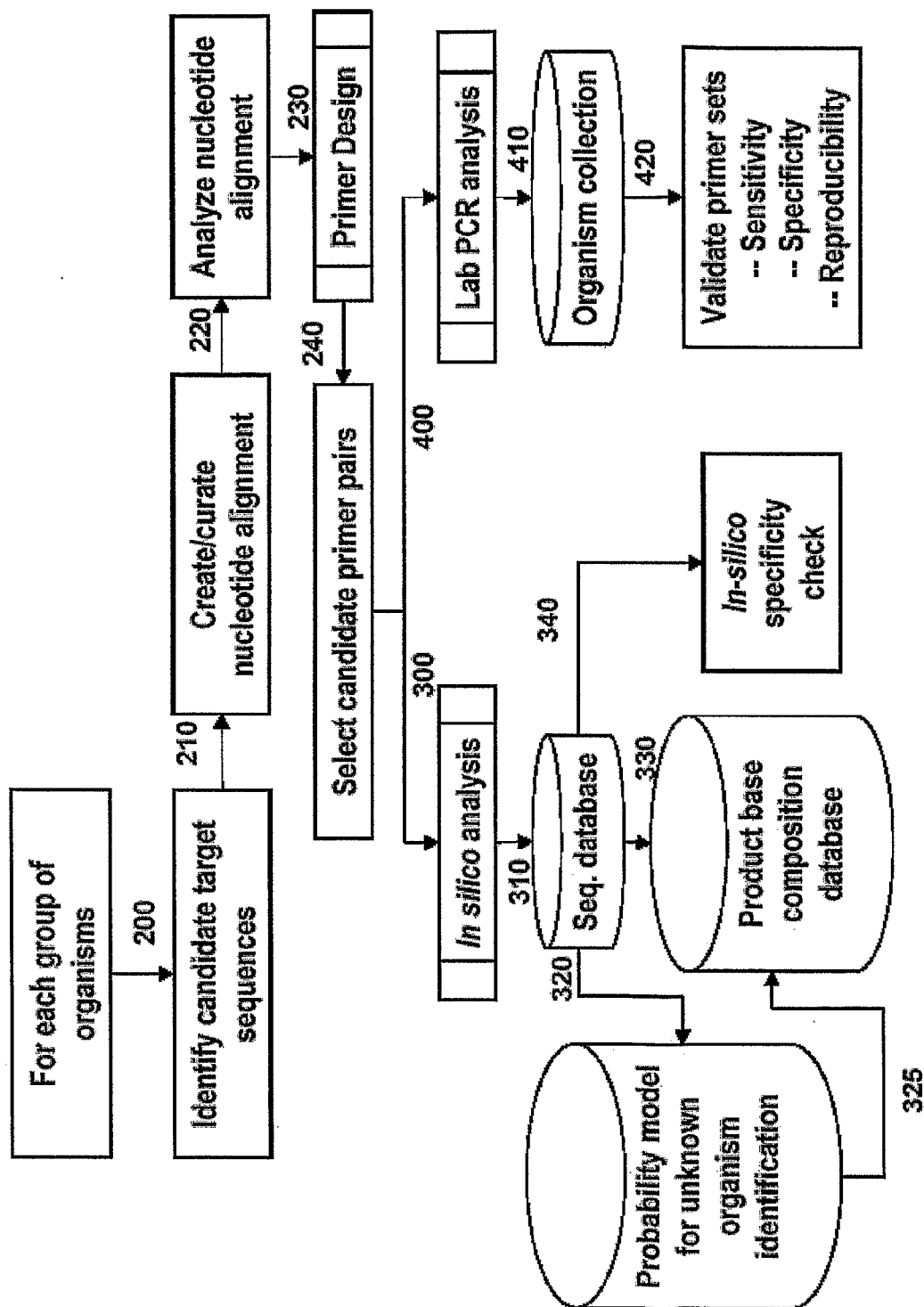


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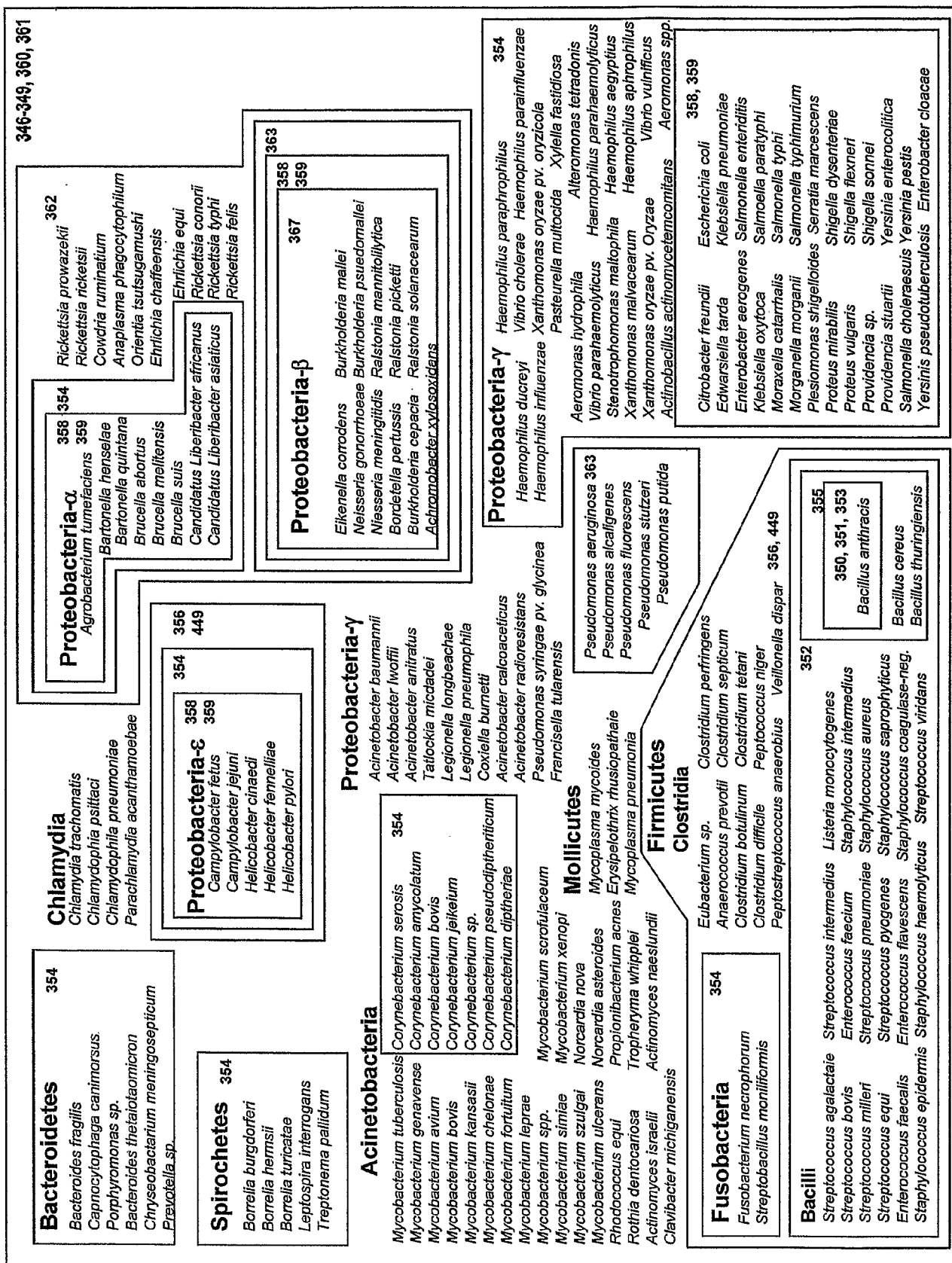
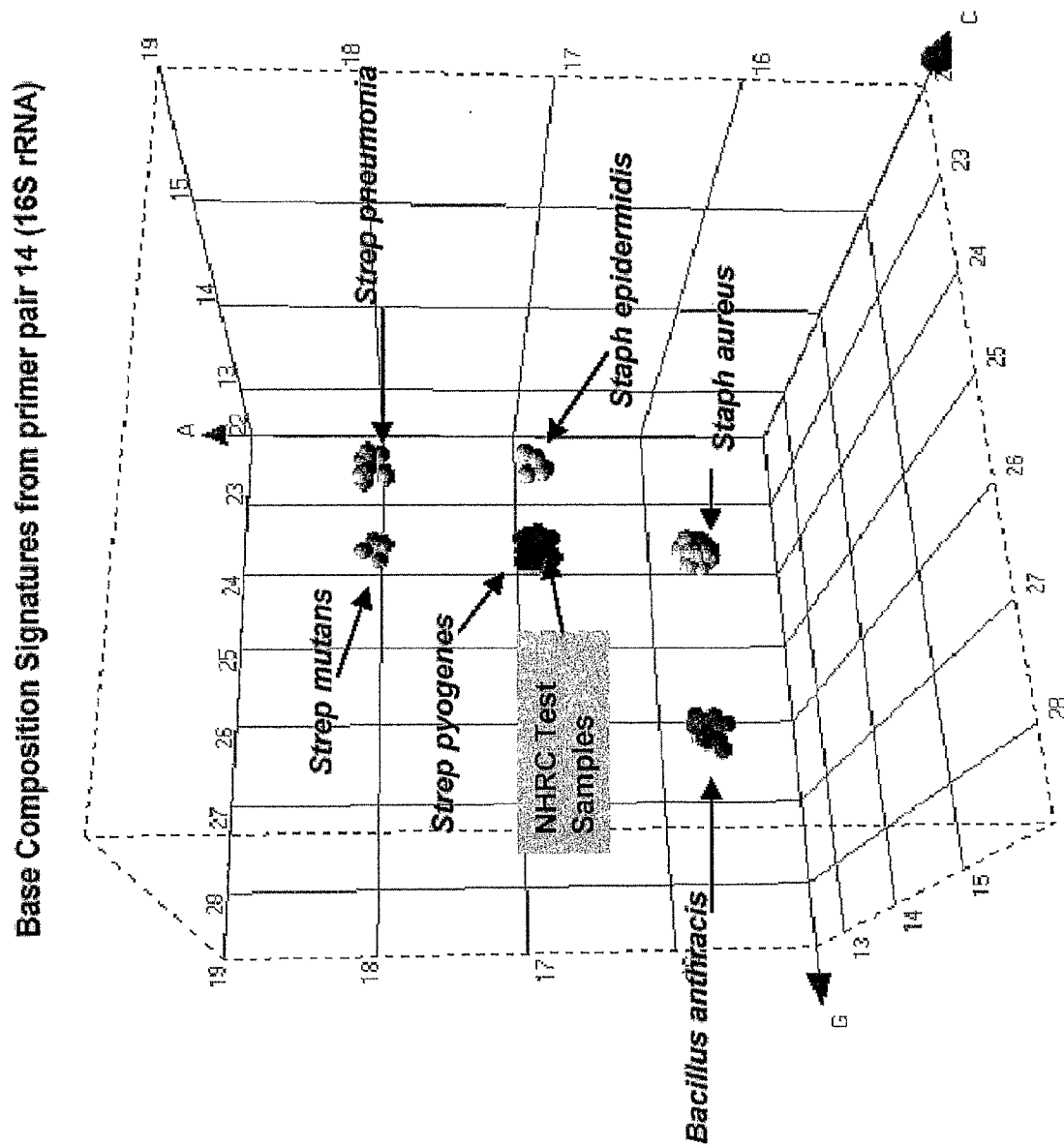
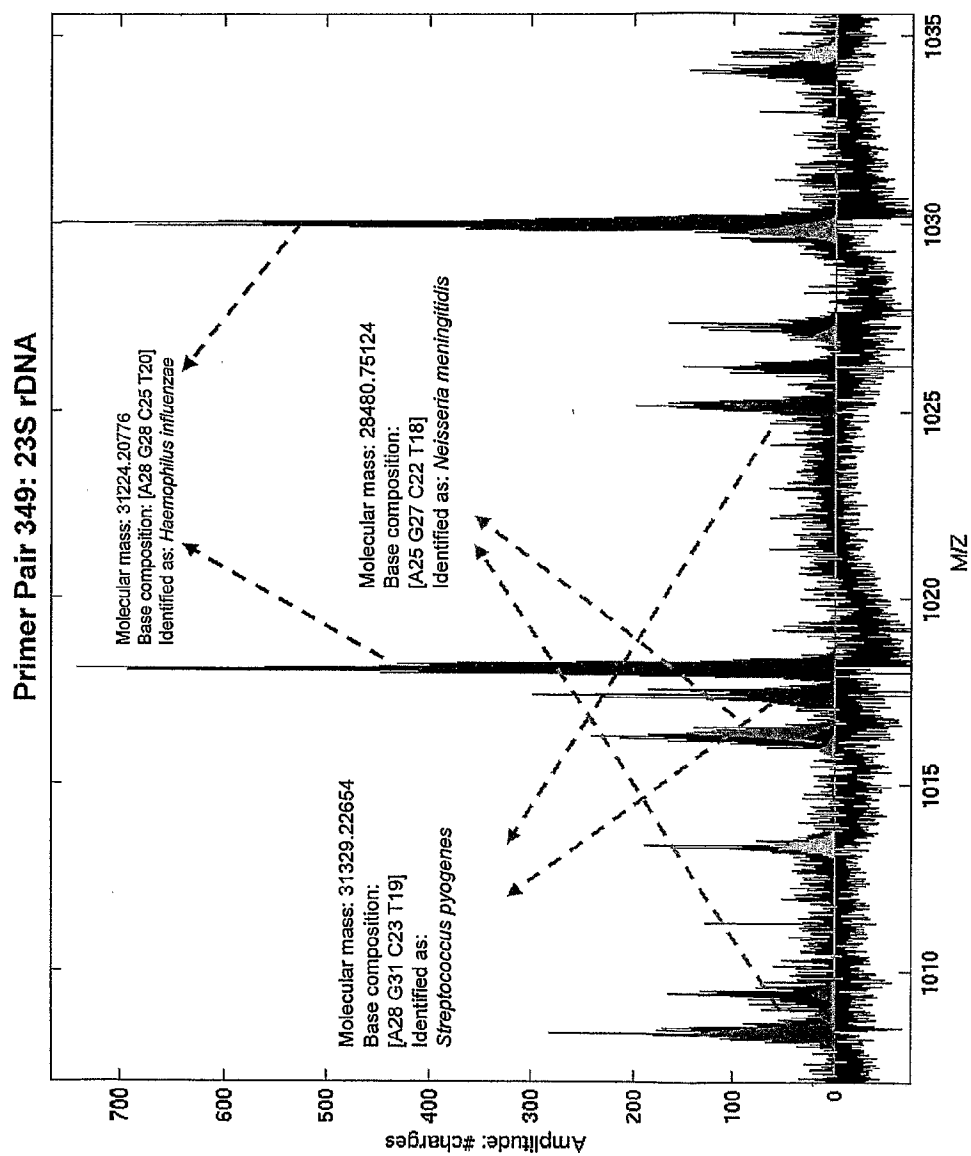


Figure 4





**Figure 5**



**Figure 6**  
**Primer 356: rpIB**

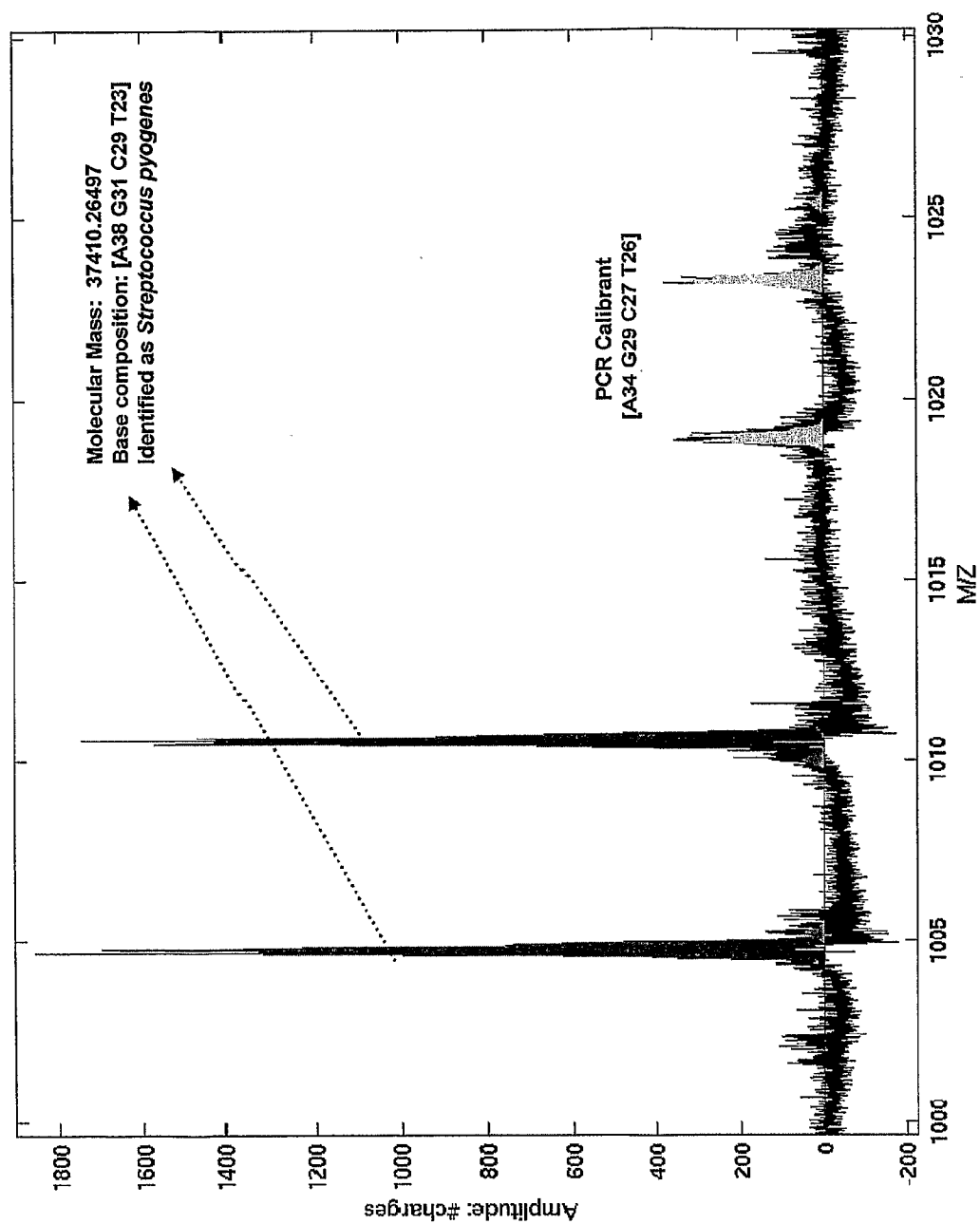


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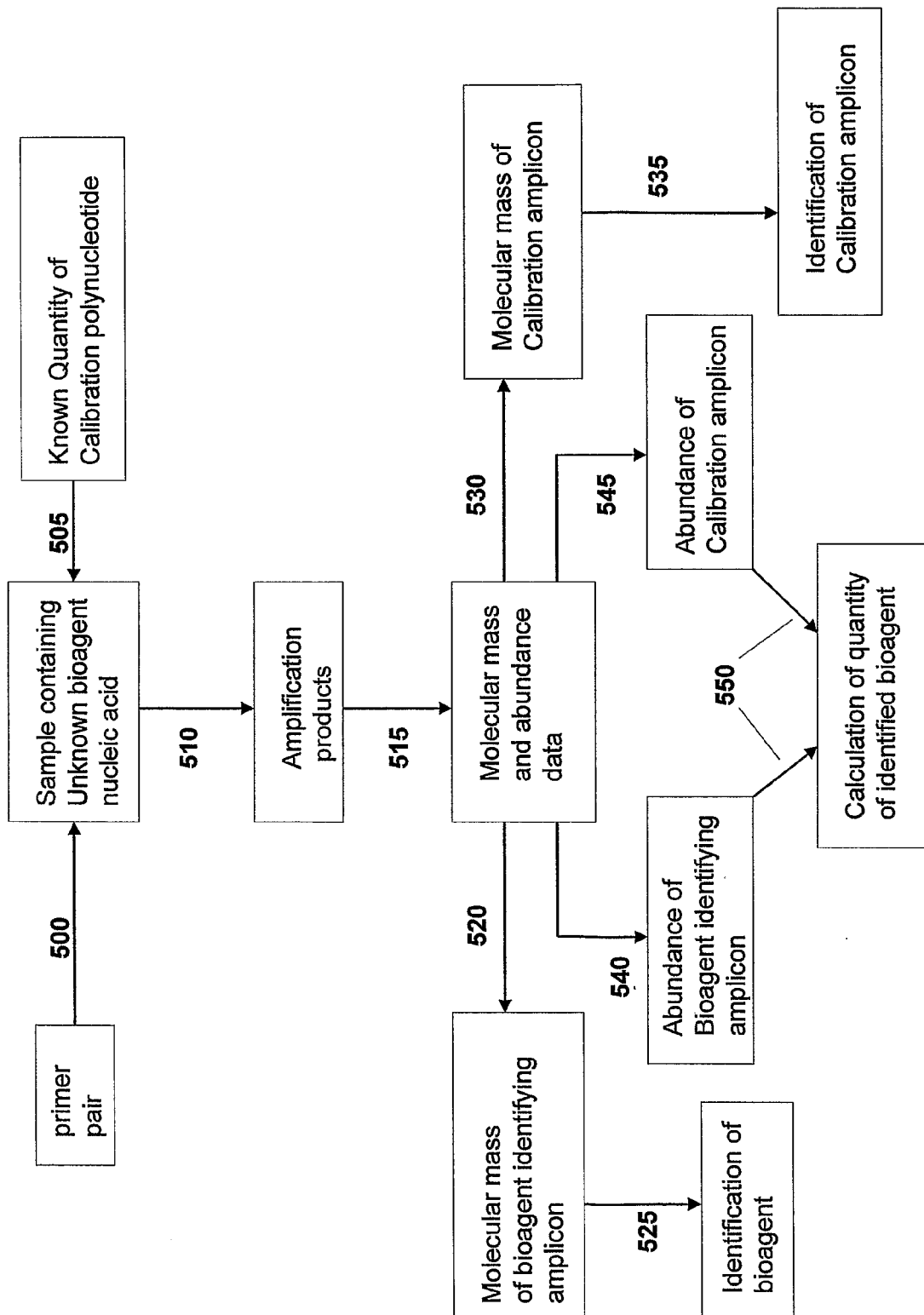
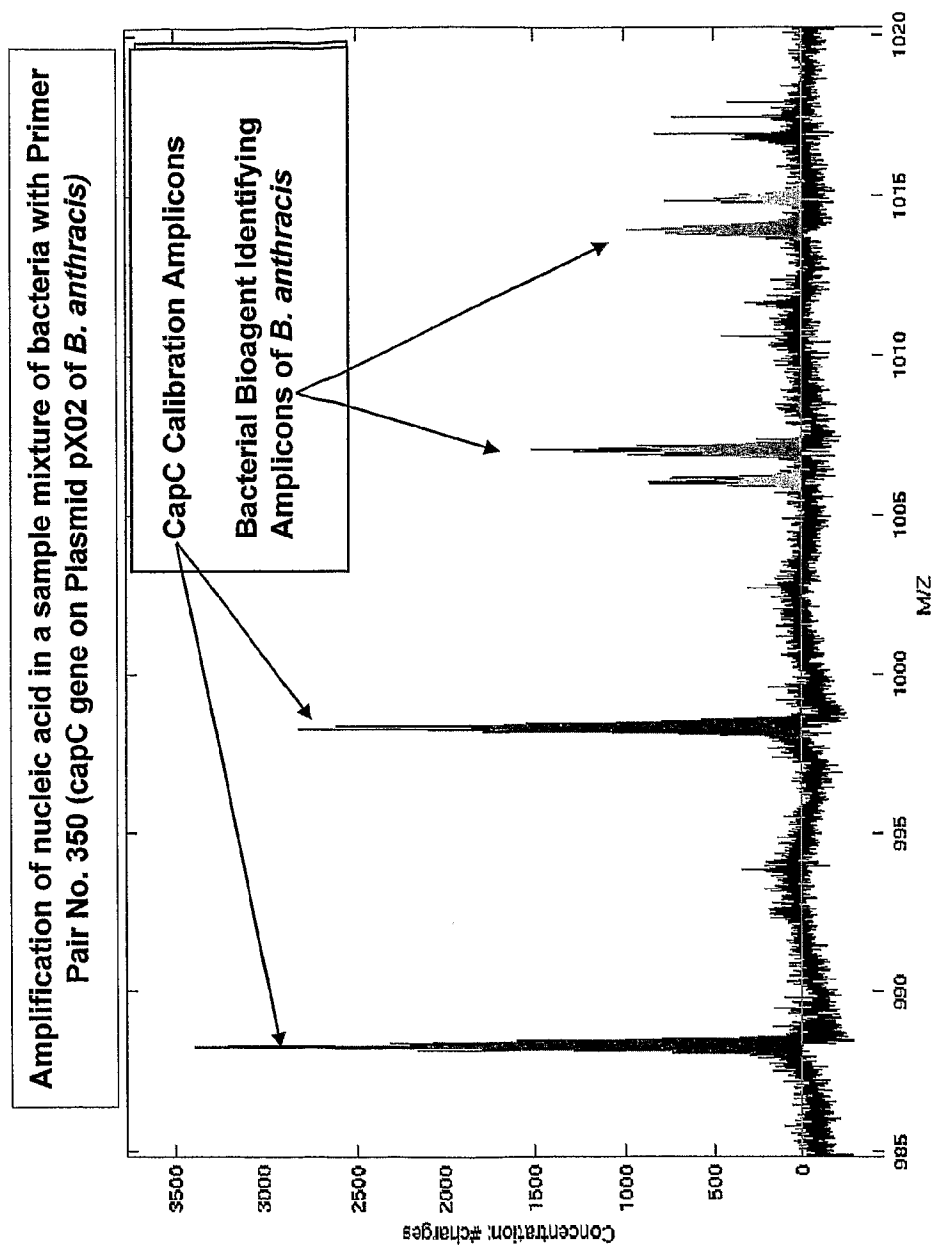


Figure 8



## SEQUENCE LISTING

<110> Isis Pharmaceuticals, Inc.  
Sampath, Rangarajan  
Hall, Thomas A.  
Ecker, David J.  
Eshoo, Mark. W.  
Massire, Christian  
Science Applications International Corporation  
Larson, Brons M.  
Leighton, Terrance

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&lt;223&gt; Primer

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&lt;213&gt; Artificial Sequence

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<223> *Bacillus anthracis*

&lt;400&gt; 722

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<223> Escherichia coli

<400> 723

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<210> 724

<211> 1647

<212> DNA

<213> Artificial Sequence

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<223> Escherichia coli

<400> 724

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&lt;210&gt; 725

&lt;211&gt; 1935

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

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&lt;223&gt; Escherichia coli

&lt;400&gt; 725

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&lt;210&gt; 726

&lt;211&gt; 2673

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Escherichia coli

&lt;400&gt; 726

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<220>  
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<400> 727  
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<210> 728  
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 <213> Artificial Sequence

<220>  
 <223> Bacillus anthracis

<400> 728

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&lt;210&gt; 729

&lt;211&gt; 822

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

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&lt;400&gt; 729

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<223> Escherichia coli

<400> 730

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<400> 731

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<220>  
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<220>  
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&lt;210&gt; 736

&lt;211&gt; 1770

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Escherichia coli

&lt;400&gt; 736

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&lt;210&gt; 737

&lt;211&gt; 3699

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Yersinia pestis*

&lt;400&gt; 737

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&lt;223&gt; Clostridium botulinum

&lt;400&gt; 738

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Staphylococcus aureus*

&lt;400&gt; 739

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&lt;211&gt; 1832

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&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Acinetobacter baumannii*

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<220>

<223> *Acinetobacter baumanii*

<400> 741

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<211> 344

<212> DNA

<213> Artificial Sequence

<220>

<223> *Acinetobacter baumanii*

<400> 742

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tgTgaagata actacgacgg ttcggaacgt atccctgaag tacttccgaTc acgtgttcca 300
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<210> 743

<211> 909

<212> DNA

<213> Artificial Sequence

<220>

<223> *Acinetobacter baumanii*

<400> 743

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aaatacatca acgaaggTaa aaaccatctc aacgaaatct tccatttTcaTc agctgatgct 420
gacaacggTa ttgctgtaga agttgcattg caatggaacg atagtTaccTc agaaaattgt 480

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cgctgtttca caaacaacat tccacaaaaa gatggtggtg cgcacttagc aggtttccgc 540
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<210> 744

<211> 1430

<212> DNA

<213> Artificial Sequence

<220>

<223> *Acinetobacter baumanii*

<400> 744

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<210> 745

<211> 3609

<212> DNA

<213> Artificial Sequence

<220>

<223> Concatenation of *C. jejuni* genes

<220>

<221> misc\_feature

<222> 478-527, 1005-1054, 1457-1506, 2014-2063, 2562-2611, 3071-3120

<223> n = A,T,C or G

<400> 745

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gaaagaaaaa taagagaagt gacaggtttt gaataactg tggctgagga tttaatcga 180

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3609

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&lt;210&gt; 746

&lt;211&gt; 1214

<212> DNA  
<213> Artificial Sequence

<220>  
<223> Bordetella pertussis

<400> 746  
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gggtgcgtg cggccatcga gcaggcgcag cagcgaagat ttgccgcgc cgttgccgcc 180  
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taagtggggc aaacgggtgt tccagggaag cagcgcactc acgttggtat ctaaggggcg 1140  
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<210> 747  
<211> 925  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Burkholderia mallei

<400> 747  
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acgcgcaccg gatgtcctcg ccggtgccgc gtgacggacg acgcttgctt cggcgcgcgc 180  
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<210> 748  
<211> 713  
<212> DNA  
<213> Artificial Sequence

<220>



<223> *Bacillus subtilis*

<400> 748

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ttgaaggtga tgcacttgcc atctgccgtg cgaacctttg gottagaac a gccgaccgca 660
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<210> 749

<211> 828

<212> DNA

<213> Artificial Sequence

<220>

<223> *Clostridium perfringens*

<400> 749

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ggctgcccggt cccgtcctcg ggtgtgtcgc ttgagcctat cggcaacggt aggcctagat 660
agatgattgt caaatacaga actcggttta tagacttato tcgtatttat aaaacactag 720
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<210> 750

<211> 777

<212> DNA

<213> Artificial Sequence

<220>

<223> *Escherichia coli*

<400> 750

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<210> 751

<211> 834

<212> DNA

<213> Artificial Sequence

<220>

<223> Rickettsia prowazekii

<400> 751

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 gaattgacat tatggaattht gttattataa ttgtaattat attgtgtgta caataattac 180  
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 gtaataaccg cagagttatt gcgagtaact gaaaaagta tggcaatcta gaaaataaat 720  
 cagattctgt ggatttttag tgttccctcg caatgacgaa aataatacac gtatagatta 780  
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<210> 752

<211> 783

<212> DNA

<213> Artificial Sequence

<220>

<223> Staphylococcus aureus

<400> 752

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 ggctatgaaa caaatgttga aaatggtcgt atattttttg aaggagacgc aagtgcatt 720  
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<210> 753

<211> 1086

<212> DNA

<213> Artificial Sequence

<220>

<223> Vibrio cholerae

<400> 753

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&lt;210&gt; 754

&lt;211&gt; 369

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Coxiella burnetii*

&lt;400&gt; 754

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tcgctcaat 369

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&lt;210&gt; 755

&lt;211&gt; 1317

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Acinetobacter baumannii*

&lt;400&gt; 755

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&lt;210&gt; 756

&lt;211&gt; 4932

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Rickettsia prowazekii

&lt;400&gt; 756

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&lt;210&gt; 757

&lt;211&gt; 1311

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Rickettsia prowazekii

&lt;400&gt; 757

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&lt;210&gt; 758

&lt;211&gt; 882

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Vibrio cholerae*

&lt;400&gt; 758

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&lt;210&gt; 759

&lt;211&gt; 1095

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Francisella tularensis*

&lt;400&gt; 759

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&lt;210&gt; 760

&lt;211&gt; 1020

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Francisella tularensis*

&lt;400&gt; 760

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&lt;210&gt; 761

&lt;211&gt; 840

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> *Shigella flexneri*

&lt;400&gt; 761

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 <211> 503  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Campylobacter jejuni

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<210> 763  
 <211> 2118  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Concatenation of A. baumannii genes

<220>  
 <221> misc\_feature  
 <222> 447-486, 778-817, 1162-1201, 1495-1534, 1928-1967, 2115-2118  
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&lt;210&gt; 764

&lt;211&gt; 276

&lt;212&gt; DNA

<213> *Acinetobacter baumannii*

&lt;400&gt; 764

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&lt;210&gt; 765

&lt;211&gt; 9610

&lt;212&gt; DNA

<213> *Yersinia pestis*

&lt;400&gt; 765

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&lt;211&gt; 102

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 766

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&lt;210&gt; 767

&lt;211&gt; 94

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 767

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&lt;210&gt; 768

&lt;211&gt; 108

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 768

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&lt;211&gt; 108

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 769

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&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 770

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&lt;210&gt; 771

&lt;211&gt; 117

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 771

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&lt;210&gt; 772

&lt;211&gt; 100

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 772

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&lt;210&gt; 773

&lt;211&gt; 112

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Calibration Polynucleotide

&lt;400&gt; 773

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&lt;210&gt; 774

&lt;211&gt; 100

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

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&lt;223&gt; Calibration Polynucleotide

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